

12-22-01 414 new COMP 27 DEC 2000

FORM PTO-1390 (Modified) (REV 11-98)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER ACY33427-00 <i>Box 509</i>	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5) 09/720560	
INTERNATIONAL APPLICATION NO. PCT/US99/14375		INTERNATIONAL FILING DATE 015 Sept 1999		PRIORITY DATE CLAIMED 26 June 1998	
TITLE OF INVENTION Novel Antigens of Helicobacter Pylori					
APPLICANT(S) FOR DO/EO/US FULGINITI, James Peter; FISKE, Michael James; BILTS, Deborah Ann					
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:					
<ul style="list-style-type: none">1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.3. <input type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.5. <input type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371 (c) (2))<ul style="list-style-type: none">a. <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).b. <input type="checkbox"/> has been transmitted by the International Bureau.c. <input checked="" type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).6. <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).7. <input type="checkbox"/> A copy of the International Search Report (PCT/ISA/210).8. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))<ul style="list-style-type: none">a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).b. <input type="checkbox"/> have been transmitted by the International Bureau.c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.d. <input checked="" type="checkbox"/> have not been made and will not be made.9. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).10. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).11. <input checked="" type="checkbox"/> A copy of the International Preliminary Examination Report (PCT/IPEA/409).12. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).					
Items 13 to 20 below concern document(s) or information included:					
<ul style="list-style-type: none">13. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.14. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.15. <input type="checkbox"/> A FIRST preliminary amendment.16. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.17. <input type="checkbox"/> A substitute specification.18. <input type="checkbox"/> A change of power of attorney and/or address letter.19. <input checked="" type="checkbox"/> Certificate of Mailing by Express Mail20. <input checked="" type="checkbox"/> Other items or information: sequence listing					
CERTIFICATION UNDER 37 CFR §1.10 I hereby certify that this paper and the documents referred to as enclosed therein are being deposited with the United States Postal Service on the date written below in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number EL704979485US addressed to the Assistant Commissioner for Patents, Washington, DC 20231.					
Date <i>12/21/00</i> Name: <i>Joan Beutagun</i>					

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5) <div style="font-size: 24pt; font-weight: bold; text-align: center;">09/720560</div>		INTERNATIONAL APPLICATION NO. <div style="text-align: center;">PCT/US99/14375</div>		ATTORNEY'S DOCKET NUMBER <div style="text-align: center;">ACY33427-00</div>	
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21. The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :				CALCULATIONS PTO USE ONLY	
<input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1,000.00 <input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$860.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$710.00 <input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$690.00 <input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00				<div style="border: 1px solid black; width: 100%; height: 100%;"></div>	
ENTER APPROPRIATE BASIC FEE AMOUNT =					
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).				\$0.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	56 - 20 =	36	x \$18.00	\$648.00	
Independent claims	15 - 3 =	12	x \$80.00	\$960.00	
Multiple Dependent Claims (check if applicable) <input type="checkbox"/>				\$0.00	
TOTAL OF ABOVE CALCULATIONS =				\$2,468.00	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) (check if applicable). <input type="checkbox"/>				\$0.00	
SUBTOTAL =				\$2,468.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).				\$0.00	
TOTAL NATIONAL FEE =				\$2,468.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). <input type="checkbox"/>				\$0.00	
TOTAL FEES ENCLOSED =				\$2,468.00	
				Amount to be:	\$
				refunded	
				charged	\$

☐ A check in the amount of _____ to cover the above fees is enclosed.

☒ Please charge my Deposit Account No. **01-1300** in the amount of **\$2,468.00** to cover the above fees.
 A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **01-1300** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE
 Darryl L. Webster
 NAME
 34,276
 REGISTRATION NUMBER
 12-21-00
 DATE

09/720560
528 Rec'd PCT/PTO 21 DEC 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re of Application of: James Peter Fulginiti et al.

Serial No.:

Group No.:

Filed:

Examiner:

For:

Novel Antigens of Helicobacter Pylori

Assistant Commissioner of Patents
Washington, DC 20231

Sir:

STATEMENT UNDER 37 C.F.R. §1.821(f)

I hereby certify that the content of the paper copy required by 37 C.F.R. §1.821(c) and the computer readable form of the Sequence Listings required by 37 C.F.R. §1.821(e) for the above-captioned patent application are the same.

Respectfully submitted,



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CERTIFICATION UNDER 37 CFR §1.10(a)

I hereby certify that this paper and the documents referred to as enclosed therein are being deposited with the United States Postal Service on the date written below in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number **EL704979485US** addressed to the Assistant Commissioner for Patents, Washington, DC 20231.

12/21/00
Date

Joan Butagno

Rec'd PCT/PTO 01 OCT 2001

AC 3427 PCT/US
PATENTS

#3

UNDER THE PATENT COOPERATION TREATY

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Applicant(s): AMERICAN CYANAMID COMPANY Customer Number: 25291

U.S. Appl'n No. 09/720,560

Int'l Appl'n No.: PCT/US 99/14375

Int'l Filing Date: 25 JUNE 1999

For: Novel Antigens of Helicobacter Pylori

October 1, 2001

Statement under 37 CFR 1.821 for Sequence Listing

Commissioner for Patents
Box PCT
United States Patent and Trademark Office
Washington, Dc 20231
Attn: John L. Anderson

S I R:

I hereby certify that the content of the paper copy required by 1.821(c) and the computer readable form of the Sequence Listing required by 1.821(e) for the above-captioned application are the same. In addition, this revised Sequence Listing does not include new matter.

Respectfully submitted,

Phone: (973)-683-2159

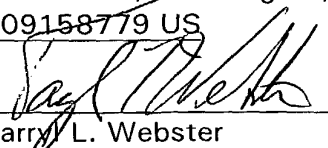
Attorney for Applicants
Darryl L. Webster, Reg. No. 34,276

~~~~~  
**CERTIFICATION UNDER 37 CFR 1.10**

I hereby certify that this paper and the documents referred to as enclosed therein are being hand deposited with the United States Postal Service on the date written below with sufficient postage as for Express Mail delivery in an envelope addressed to the Commissioner for Patents, Box PCT, Washington, DC 20231 with Express Mail Label No. EL909158779 US

1 October 2001

Date

  
Darryl L. Webster

## PETITION, Part B

If an additional extension of time is required, please consider this a petition therefor.

## PETITION, Part C

## Method of Payment

Charge the Five-month Extension of Time to  
Deposit Account No. 01-1425.

**A duplicate of this transmittal is attached.**

## PETITION, Part D

## Instructions as to Overpayment

Credit any overpayment to Deposit Account No. 01-1425.

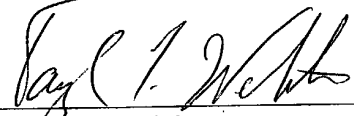
## PETITION, Part E

## Authorization to Charge Additional Fees

If any additional extension and/or fees is/are required, charge  
Account No. 01-1425.

10/04/2001 HIKAYPAGH 00000111 011425 09720560  
01 FC:128 1960.00 CH

Respectfully submitted,  
Fulginiti et al



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## **NOVEL ANTIGENS of *HELICOBACTER PYLORI***

### **Field of the Invention**

- 5                   The present invention relates to novel nucleic acids and polypeptides relating to *Helicobacter pylori*. The nucleic acid sequences and polypeptides are useful for diagnostic and therapeutic purposes.

### **Background of the Invention**

- 10                   *Helicobacter pylori* (*H. pylori*) is a gram-negative, S-shaped, microaerophilic bacterium that was discovered and cultured from a human gastric biopsy specimen infection. [Warren, J.R. et al., *Lancet*, 1: 1273-1275, (1983) ; and Marshall et al., *Microbios Lett*, 25: 83-88, (1984)]. *H. pylori* has been strongly linked to chronic gastritis and duodenal ulcer disease. [Rathbone et al., *Gut*, 27: 635-641, 15 (1986)]. Additional evidence has developed for an etiological role of *H. pylori* in non-ulcer dyspepsia, gastric ulcer disease, and gastric adenocarcinoma. [Blaser, M.J., *Trends Microbiol.*, 1: 255-260, (1993)]. *H. pylori* colonizes the human gastric mucosa, establishing an infection that usually persists for many years. About 30-50% of the human population appear to be chronically infected. [Rainer, H. et al, *Biologicals*, 25: 20 175-177, (1997)]. The current recommended treatment for chronic *H. pylori* infection is multiple antibiotic treatment combined with a proton pump inhibitor, or with bismuth salts. However, this treatment may not fully resolve the infection and resistance to antibiotics can occur. *H. pylori* infection in humans induces a strong local and systemic immune response; however, the immune response is often unable to clear the infection.
- 25                   Accordingly, work has led to developing potential *H. pylori* vaccines. Various antigens, proteins and genes have been reported in this area. [See Bolin et al., PCT Application No. 96/38475, filed June 3, 1996; Doidge et al., PCT Application No. 96/33220, filed April 19, 1996; Clancy et al., PCT Application No. 96/25430, filed February 15, 1996; Chan et al., PCT Application No. 96/12965, filed October 19, 1995; Byrne et al., PCT 30 Application No. 96/01273, filed July 3, 1995; Alemohammad M.M., in U.S. Patent 5,262,156, filed August 12, 1991; Allan et al., PCT Application No. 97/03359, filed June 28, 1996; and Dettmar et al., German Patent Application 195235554 A1 based on Great Britain Application No. 9413074.] As an alternative to potential vaccines and

diagnostic currently provided in the art referenced above, novel methods of curing or preventing *H. pylori* infection through the use of novel *H. pylori* proteins, as well as genes encoding such proteins are described herein. Proteins were selected for use as an antigen and/or vaccine candidate based on the following criteria: (i) the antigen is  
5 located on the bacterial surface, (ii) the antigen is conserved among *H. pylori* clinical isolates, (iii) the antigen elicits functional antibodies, (iv) the antigen is able to confer protection to vaccinated mice from challenge with a live organism.

#### **Summary of the Invention**

10 This invention relates to novel *H. pylori* bacterial surface proteins and nucleic acid sequences encoding therefor, in particular novel *H. pylori* bacterial surface proteins having molecular weights of approximately 75, 77, and 79 kilo daltons (kDa). The mature processed forms of these proteins share a common amino-terminal amino acid  
15 diagnostic and therapeutic utility for *H. pylori* and other *Helicobacter* species. They can be used to detect the presence of *H. pylori* and other *Helicobacter* species in a sample, and to screen compounds for the ability to interfere with the *H. pylori* life cycle or to inhibit *H. pylori* infection. More specifically, this invention includes embodiments relating to isolated nucleic acid sequences corresponding to the entire coding sequences  
20 of *H. pylori* surface proteins or portions thereof, nucleic acids capable of binding mRNA from *H. pylori* surface proteins and methods for producing *H. pylori* surface proteins or portions thereof using peptide synthesis and recombinant techniques. Additional embodiments are also directed to antigenic and vaccine compositions based on agents prepared from the proteins and nucleic acids of this invention and methods for treatment  
25 and prevention of *H. pylori* infections employing such compositions.

#### **Brief Description of the Drawings**

Figure 1: Figure 1(A) depicts an SDS-PAGE gel with bands showing the 75kDa and  
30 77kDa proteins in lane 3 in comparison to Zwittergent™ 3-14 crude extract of *H. pylori* outer membrane proteins in lane 2 and molecular weight standards in lane 1. Figure 1(B) depicts a Western blot of monoclonal antibody 64-27 with the co-purified 75kDa and 77kDa proteins from ATCC 43579, as described in Example 1. Lane 1 is the

molecular weight markers, lane 2 is the crude extract and lane 3 is the co-purified proteins.

Figure 2 depicts an electron micrograph of the surface labeling of *H. pylori* strain PBCC 1105 with mouse polyclonal antisera to a co-purified mixture of 75/77 kDa proteins, as described in Example 2.

Figure 3 depicts two graphs of Flow Cytometry analysis of strain PBCC 1105 with labeled an anti-75/77 mouse polyclonal antibody, at day 0 (Figure 3A) and day 49 (Figure 3B) following injection of mice with a mixture of 75kDa and 77kDa proteins, as described in Example 2. Profile 1 is ATCC 43579 (homologous strain); profile 2 is strain PBCC 1105; profile 3 is strain ATCC 43504; profile 4 is strain SS-1 and profile 5 is a urease-negative strain.

Figure 4 is a graph depicting the bactericidal activity of anti-75/77kDa polyclonal mouse sera, as described in Example 3, with either incomplete Freund's adjuvant or MPL™.

Figure 5: This Figure depicts the mouse protection data (in colony forming units) from the *H. pylori* SS1 experimental challenge following vaccination of mice with a mixture of the co-purified 75kDa/77kDa proteins, as described in Example 4.

Figure 6: This Figure depicts a DNA (SEQ ID NO 19) for the 75kDa gene from strain ATCC 43579.

Figure 7: This Figure depicts the predicted translated protein sequence for the DNA sequence (SEQ ID NO 21) for the 75kDa gene from strain ATCC 43579 in figure 6.

Figure 8: This Figure depicts a DNA sequence (SEQ ID NO 20) for the 79kDa gene from strain ATCC 43579.

Figure 9: This Figure depicts the predicted translated protein sequence (SEQ ID NO 22) for the DNA sequence for the 79kDa gene from strain ATCC 43579 in figure 8.



Figure 10: This Figure depicts SDS-PAGE gel bands from comparison expression experiments from Example 13 for recombinant 75kDa protein expressed from a low copy number plasmid pBAD24 and the high copy number T7 expression plasmid pRSETb. The bands show the increased amounts of protein 75 kDa expressed.

Figure 11: This Figure depicts SDS-PAGE gel bands from comparison expression experiments from Example 13 for recombinant 77kDa protein expressed from a low copy number plasmid pET17 and the high copy number T7 expression plasmid pRSETb. The bands show the increased amounts of protein 77 kDa expressed.

Figure 12: This Figure depicts SDS-PAGE gel bands from comparison expression experiments from Example 13 for recombinant 79kDa protein expressed from a low copy number plasmid pBAD24 and the high copy number T7 expression plasmid pRSETb. The bands show the increased amounts of protein 79 kDa expressed.

Figure 13: This Figure depicts mouse protection data (in colony forming units) from the *H. pylori* SS1 experimental challenge following vaccination of mice with a mixture of the co-purified 75kDa/77kDa proteins

Figure 13: This Figure depicts therapeutic effect (in colony forming units) of a mixture of the co-purified 75kDa/77kDa proteins when vaccinating mice after infection with *H. pylori* SS1. Experiments included intragastric vaccination and a subcutaneous vaccination.

### **Detailed Description of the Invention**

One aspect of the present invention provides an isolated, substantially purified *H. pylori* polypeptide selected from the group consisting of (i) a polypeptide having a molecular weight of about 75 kDa; (ii) a polypeptide having a molecular weight of about 77 kDa; and (iii) a polypeptide having a molecular weight of about 79 kDa; wherein the mature processed form of each polypeptide has a starting sequence consisting essentially of EDDGFYTSVG YQIG EAAQMV (SEQ. ID NO.7). The present invention also relates to isolated polypeptides. Preferred embodiments of the invention relate to an isolated

polypeptide of *H. pylori* selected from the group consisting of (i) a polypeptide having a molecular weight of about 75 kDa and having the amino acid sequence of SEQ. ID NO.1 or SEQ ID NO. 19; (ii) a polypeptide having a molecular weight of about 77 kDa and having the amino acid sequence of SEQ. ID NO.2; and (iii) a polypeptide having a molecular weight of about 79 kDa and having the amino acid sequence of SEQ. ID NO.3 or SEQ ID NO. 20. Preferably, the polypeptides of this invention have antigenic properties, such as being reactive with *H. pylori* antibodies. Antigens can be based on the isolated polypeptides sequences, or allelic or other variants thereof, which are biological equivalents. Suitable biological equivalents have about 70 to about 80%, and most preferably at least about 90%, similarity to one of the amino acid sequences referred to above, or to a portion thereof, provided the equivalent is capable of eliciting substantially the same antigenic properties as the isolated polypeptide sequences specified hereinabove.

The biological equivalents are obtained by generating variants and modifications to the isolated polypeptides of this invention. These variants and modifications to the isolated polypeptides are obtained by altering the amino acid sequences by insertion, deletion or substitution of one or more amino acids. The polypeptides are then selected for use as an antigen and/or vaccine candidate based on the following criteria: (i) the antigen is located on the bacterial surface, (ii) the antigen is conserved among *H. pylori* clinical isolates, (iii) the antigen elicits functional antibodies, (iv) the antigen is able to confer protection to vaccinated mice from challenge with a live organism. Modifying the amino acid, for example by substitution, the amino acids of the protein to create a polypeptide having substantially the same or improved qualities. The amino acid changes are achieved by changing the codons of the nucleic acid sequence. It is known that such polypeptides can be obtained based on substituting certain amino acids for other amino acids in the polypeptide structure in order to modify or improve antigenic or immunogenic activity (see, e.g. Kyte and Doolittle, 1982, Hopp, US Patent 4,554,101, each incorporated herein by reference). For example, through substitution of alternative amino acids, small conformational changes may be conferred upon a polypeptide which result in increased activity or enhanced immune response. Alternatively, amino acid substitutions in certain polypeptides may be utilized to provide residues which may then be linked to other

molecules to provide peptide-molecule conjugates which retain sufficient antigenic properties of the starting polypeptide to be useful for other purposes. For example, a selected polypeptide of the present invention may be bound to a solid support in order to have particular advantages for diagnostic applications.

5

One can use the hydropathic index of amino acids in conferring interactive biological function on a polypeptide, as discussed by Kyte and Doolittle (1982), wherein it is found that certain amino acids may be substituted for other amino acids having similar hydropathic indices and still retain a similar biological activity. Alternatively, substitution of like amino acids may be made on the basis of hydrophilicity, particularly where the biological function desired in the polypeptide to be generated is intended for use in immunological embodiments. US Patent 4,554,101, which states that the greatest local average hydrophilicity of a "protein," as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity. Accordingly, it is noted that substitutions can be made based on the hydrophilicity assigned to each amino acid.

10  
15

In using either the hydrophilicity index or hydropathic index, which assigns values to each amino acid, it is preferred to conduct substitutions of amino acids where these values are  $\pm 2$ , with  $\pm 1$  being particularly preferred, and those within  $\pm 0.5$  being the most preferred substitutions.

20

Preferable characteristics of the polypeptides of this invention include one or more of the following: (a) being a membrane protein or being a protein directly associated with a membrane; (b) capable of being separated as a protein using an SDS acrylamide (10%) gel; (c) generating antibody which exhibits bactericidal activity upon injection in a mouse; and/or (d) reducing the colonization of *H. pylori* in mice upon delivery thereto.

25

The isolated polypeptides having molecular weights of about 75, 77, and 79 kDa are particular useful in antigenic compositions for several reasons. First, using monoclonal antibodies derived by injecting mice with whole bacteria, these polypeptides were identified as closely related outer membrane proteins of *H. pylori*. Second, our analyses by a number of *in vitro* techniques indicate that the polypeptides

30

are surface localized and that they are expressed in a variety of clinically relevant strains. Third, the polyclonal antibodies generated by injecting mice with a mixture of the purified polypeptides are bactericidal. Fourth, we observed that following oral vaccination with the polypeptides admixed with cholera toxin (CT), 100% of mice  
5 challenged with *H. pylori* strain SS1 showed a statistically significant reduction in colonization as compared to non-vaccinated control mice.

Accordingly, further embodiments of this invention relate to an antigenic composition comprising (i) at least one isolated polypeptide as disclosed above and (ii)  
10 a pharmaceutically acceptable buffer, diluent, adjuvant or carrier. The antigenic composition may comprise a carrier, which in turn may be conjugated to said polypeptide. In additional embodiments, the antigenic composition may further comprise an adjuvant. Preferably, these compositions have therapeutic and prophylactic applications as vaccines in preventing and/or ameliorating *H. pylori* infection. In such  
15 applications immunologically effective amount (as discussed herein) of at least one polypeptide of this invention is employed.

The formulation of such prophylactic or therapeutic antigenic compositions is well known to persons skilled in this field. Antigenic compositions of the invention  
20 containing antigenic components (e.g., *H. pylori* polypeptide or fragment thereof or nucleic acid encoding an *H. pylori* polypeptide or fragment thereof) preferably include a pharmaceutically acceptable carrier. Suitable pharmaceutically acceptable carriers and/or diluents include any and all conventional solvents, dispersion media, fillers, solid carriers, aqueous solutions, coatings, antibacterial and antifungal agents, isotonic and  
25 absorption delaying agents, and the like. The term "pharmaceutically acceptable carrier" refers to a carrier that does not cause an allergic reaction or other untoward effect in patients to whom it is administered. Suitable pharmaceutically acceptable carriers include, for example, one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof.  
30 Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar

as any conventional media or agent is incompatible with the active ingredient, use thereof in the antigenic compositions of the present invention is contemplated.

Such antigenic compositions are conventionally administered parenterally, e.g.,  
5 by injection, either subcutaneously or intramuscularly. Methods for intramuscular immunization are described by Wolff et al. and by Sedegah et al. Other modes of administration include oral and pulmonary formulations, suppositories, and transdermal applications. Oral immunization is preferred over parenteral methods for inducing protection against infection by *H. pylori* (See Czinn et al.). Oral formulations include  
10 such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like.

The antigenic compositions of the invention can include an adjuvant, including,  
15 but not limited to aluminum hydroxide; aluminum phosphate; Stimulon™ QS-21 (Aquila Biopharmaceuticals, Inc., Worcester, MA); MPL™ (3-O-deacylated monophosphoryl lipid A; RIBI ImmunoChem Research, Hamilton, MT), IL-12 (Genetics Institute, Cambridge, MA) N-acetyl-muramyl--L-theronyl-D-isoglutamine (thr-MDP); N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as  
20 nor-MDP); N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy--ethylamine (CGP 19835A, referred to a MTP-PE); and cholera toxin. Others which may be used are non-toxic derivatives of cholera toxin, including its B subunit, and/or conjugates or genetically engineered fusions of the *H. pylori* polypeptide with cholera toxin or its B subunit, procholeraegenoid, fungal  
25 polysaccharides, including schizophyllan, muramyl dipeptide, muramyl dipeptide derivatives, phorbol esters, labile toxin of *E. coli*, non-*H. pylori* bacterial lysates, block polymers or saponins.

Preferably also, this antigenic composition or an isolated polypeptide of this  
30 invention is used in a vaccine composition for oral administration which includes a mucosal adjuvant.

In a particularly preferred aspect of this invention, an oral vaccine composition comprising an antigenic composition in association with a mucosal adjuvant, is used for the treatment or prevention of *H. pylori* infection in a human host.

5           The mucosal adjuvant can be cholera toxin; however, preferably, mucosal adjuvants other than cholera toxin which may be used in accordance with the present invention include non-toxic derivatives of cholera toxin, such as the B sub-unit (CTB), chemically modified cholera toxin, or related proteins produced by modification of the cholera toxin amino acid sequence. These may be added to, or conjugated with, the  
10   *Helicobacter* antigenic composition. The same techniques can be applied to other molecules with mucosal adjuvant or delivery properties such as *Escherichia coli* heat labile toxin (LT). Other compounds with mucosal adjuvant or delivery activity may be used such as bile; polycations such as DEAE-dextran and polyornithine; detergents such as sodium dodecyl benzene sulphate; lipid-conjugated materials; antibiotics such as  
15   streptomycin; vitamin A; and other compounds that alter the structural or functional integrity of mucosal surfaces. Other mucosally active compounds include derivatives of microbial structures such as MDP; acridine and cimetidine. QS-21, MPL™, and IL-12, which described above, may also be used

20           The *Helicobacter* antigenic composition of this invention may be delivered in the form of ISCOMS (immune stimulating complexes), ISCOMS containing CTB, liposomes or encapsulated in compounds such as acrylates or poly(DL-lactide-co-glycoside) to form microspheres of a size suited to adsorption by M cells. Alternatively, micro or nanoparticles may be covalently attached to molecules such as vitamin B12  
25   which have specific gut receptors. The *Helicobacter* isolated polypeptides of this invention may also be incorporated into oily emulsions.

          The *Helicobacter* isolated polypeptides of the present invention may be administered as the sole active immunogen in an antigenic composition. Alternatively,  
30   however, the antigenic, or vaccine, composition may include other active immunogens, including other *Helicobacter* antigens such as urease, lipopolysaccharide, Hsp60, VacA, CagA or catalase, as well as immunologically active antigens against other pathogenic species.

One of the important aspects of this invention relates to a method of inducing immune responses in a mammal comprising the step of providing to said mammal an antigenic composition of this invention. Preferred embodiments relate to a method for the treatment or prevention of *Helicobacter* infection in a human comprising administering to a human an immunologically effective amount of an antigenic composition. Immunologically effective amount, as used herein, means the administration of that amount to a mammalian host, either in a single dose or as part of a series of doses, sufficient to at least cause the immune system of the individual treated to generate a response that reduces the clinical impact of the bacterial infection. This may range from a minimal decrease in bacterial burden to prevention of the infection. Ideally, the treated individual will not exhibit the more serious clinical manifestations of the *Helicobacter* infection. The dosage amount can vary depending upon specific conditions of the individual. This amount can be determined in routine trials by means known to those skilled in the art.

Another specific aspect of the present invention relates to using a vaccine vector expressing an isolated *Helicobacter* polypeptide, or an immunogenic fragment thereof. Accordingly, in a further aspect this invention provides a method of inducing an immune response in a mammal, which comprises providing to a mammal a vaccine vector expressing at least one, or a mixture of isolated *Helicobacter* polypeptides of this invention, or an immunogenic fragment thereof. The isolated polypeptides of the present invention can be delivered to the mammal using a live vaccine vector, in particular using live recombinant bacteria, viruses or other live agents, containing the genetic material necessary for the expression of the an antigenic polypeptide or immunogenic fragment as a foreign polypeptide. Particularly, bacteria that colonize the gastrointestinal tract, such as *Salmonella*, *Shigella*, *Yersinia*, *Vibrio*, *Escherichia* and BCG have been developed as vaccine vectors, and these and other examples are discussed by Holmgren et al. (1992) and McGhee et al. (1992).

30

An additional embodiment of the present invention relates to a method of inducing an immune response in a human comprising administering to said human an amount of a DNA molecule encoding an isolated polypeptide of this invention,

optionally with a transfection-facilitating agent, where said polypeptide retains immunogenicity and, when incorporated into an antigenic composition or vaccine and administered to a human, provides protection without inducing enhanced disease upon subsequent infection of the human with *Helicobacter* pathogen, such as *H. pylori*.

5 Transfection-facilitating agents are known in the art.

The present invention also relates to an antibody, which may either be a monoclonal or polyclonal antibody, specific for antigenic polypeptides as described above. Such antibodies may be produced by methods which are well known to those skilled in the art. The antibodies of this invention can be employed in a method for the treatment or prevention of *Helicobacter* infection in mammalian hosts, which comprises administration of an immunologically effective amount of antibody, specific for antigenic polypeptide as described above.

15 It is proposed that the monoclonal antibodies of the present invention will find useful application in standard immunochemical procedures, such as ELISA and western blot methods, as well as other procedures which may utilize antibodies specific to *H. pylori* proteins. While ELISAs are preferred, it will be readily appreciated that such assays include RIAs and other non-enzyme linked antibody binding assays or procedures. Additionally, it is proposed that monoclonal antibodies specific to the particular *H. pylori* protein or polypeptides may be utilized in other useful applications. For example, their use in immunoadsorbent protocols may be useful in purifying native or recombinant *H. pylori* proteins or variants thereof.

25 It also is proposed that the disclosed *H. pylori* polypeptides of the invention will find use as antigens for raising antibodies and in immunoassays for the detection of anti-75/77 kDa antigen-reactive antibodies. In a variation on this embodiment, samples suspected of containing *H. pylori* may be screened, in immunoassay format, for reactivity against antibodies specific for 75, 77 and 79 kDa polypeptides of this invention. Results from such analyses may then be used to determine the presence of *H. pylori* and potential infection.



Diagnostic immunoassays include direct culturing of bodily fluids or tissue, either in liquid culture or on a solid support such as nutrient agar. A typical assay involves collecting a sample of bodily fluid from a patient and placing the sample under conditions optimum for growth of the pathogen. The determination can then be made as to whether the microbe exists in the sample. Further analysis can be carried out to determine the hemolyzing properties of the microbe.

Immunoassays encompassed by the present invention include, but are not limited to those described in U.S. Patent No. 4,367,110 (double monoclonal antibody sandwich assay) and U.S. Patent No. 4,452,901 (western blot), which U.S. Patents are incorporated herein by reference. Other assays include immunoprecipitation of labeled ligands and immunocytochemistry, both *in vitro* and *in vivo*.

Immunoassays, in their most simple and direct sense, are binding assays. Certain preferred immunoassays are the various types of enzyme linked immunosorbent assays (ELISAs) and radioimmunoassays (RIAs) known in the art. Immunohistochemical detection using tissue sections is also particularly useful. However, it will be readily appreciated that detection is not limited to such techniques, and western blotting, dot blotting, FACS analyses, and the like may also be used.

In one exemplary ELISA, the anti-75/77 kDa antibodies of the invention are immobilized onto a selected surface exhibiting protein affinity, such as a well in a polystyrene microtiter plate. Then, a test composition suspected of containing the desired antigen, such as a clinical sample, is added to the wells. After binding and washing to remove non-specifically bound immune complexes, the bound antigen may be detected. Detection is generally achieved by the addition of another antibody, specific for the desired antigen, that is linked to a detectable label. This type of ELISA is a simple "sandwich ELISA." Detection may also be achieved by the addition of a second antibody specific for the desired antigen, followed by the addition of a third antibody that has binding affinity for the second antibody, with the third antibody being linked to a detectable label.

In another exemplary ELISA, the samples suspected of containing an *H. pylori* polypeptide are immobilized onto the well surface and then contacted with the anti-75/77 kDa antibodies. After binding and appropriate washing, the bound immune complexes are detected. Where the initial antigen specific antibodies are linked to a  
5 detectable label, the immune complexes may be detected directly. Again, the immune complexes may be detected using a second antibody that has binding affinity for the first antigen specific antibody, with the second antibody being linked to a detectable label.

Further methods include the detection of primary immune complexes by a two  
10 step approach. A second binding ligand, such as an antibody, that has binding affinity for the primary antibody is used to form secondary immune complexes, as described above. After washing, the secondary immune complexes are contacted with a third binding ligand or antibody that has binding affinity for the second antibody, again under conditions effective and for a period of time sufficient to allow the formation of immune  
15 complexes (tertiary immune complexes). The third ligand or antibody is linked to a detectable label, allowing detection of the tertiary immune complexes thus formed. This system may provide for signal amplification if desired.

Competition ELISAs are also possible in which test samples compete for  
20 binding with known amounts of labeled antigens or antibodies. The amount of reactive species in the unknown sample is determined by mixing the sample with the known labeled species before or during incubation with coated wells. (Antigen or antibodies may also be linked to a solid support, such as in the form of beads, dipstick, membrane or column matrix, and the sample to be analyzed applied to the immobilized antigen or  
25 antibody). The presence of reactive species in the sample acts to reduce the amount of labeled species available for binding to the well and thus reduces the ultimate signal.

Irrespective of the format employed, ELISAs have certain features in common, such as coating, incubating or binding, washing to remove non-specifically bound  
30 species, and detecting the bound immune complexes. These are described below.

In coating a plate with either antigen or antibody, one will generally incubate the wells of the plate with a solution of the antigen or antibody, either overnight or for a

WO 00/00614

PCT/US99/14375

specified period. The wells of the plate will then be washed to remove incompletely absorbed material. Any remaining available surfaces of the wells are then "coated" with a nonspecific protein that is antigenically neutral with regard to the test antisera. These include bovine serum albumin (BSA), casein and solutions of milk powder. The coating  
5 allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific binding of antisera onto the surface.

After binding of antigenic material to the well, coating with a non-reactive material to reduce background, and washing to remove unbound material, the  
10 immobilizing surface is contacted with the antisera or clinical or biological extract to be tested in a manner conducive to immune complex (antigen/antibody) formation. Such conditions preferably include diluting the antisera with diluents such as BSA, bovine gamma globulin (BGG) and phosphate buffered saline (PBS)/Tween. These added agents also tend to assist in the reduction of nonspecific background. The layered  
15 antisera is then allowed to incubate for from 2 to 4 hours, at temperatures preferably on the order of 25° to 27°C. Following incubation, the antisera-contacted surface is washed so as to remove non-immunocomplexed material. A preferred washing procedure includes washing with a solution such as PBS/Tween, or borate buffer.

20 Following formation of specific immunocomplexes between the test sample and the bound antigen, and subsequent washing, the occurrence and even amount of immunocomplex formation may be determined by subjecting same to a second antibody having specificity for the first. Of course, in that the test sample will typically be of human origin, the second antibody will preferably be an antibody having specificity in  
25 general for human IgG. To provide a detecting means, the second antibody will preferably have an associated enzyme that will generate a color development upon incubating with an appropriate chromogenic substrate. Thus, for example, one will desire to contact and incubate the antisera-bound surface with a urease or peroxidase-conjugated anti-human IgG for a period of time and under conditions which favor the  
30 development of immunocomplex formation (e.g., incubation for 2 hours at room temperature in a PBS-containing solution such as PBS-Tween).

After incubation with the second enzyme-tagged antibody, and subsequent to washing to remove unbound material, the amount of label is quantified by incubation with a chromogenic substrate such as urea and bromocresol purple or 2,2'-azino-di-(e-ethyl-benzthiazoline-6-sulfonic acid [ABTS] and H<sub>2</sub>O<sub>2</sub>, in the case of peroxidase as the enzyme label. Quantification is then achieved by measuring the degree of color generation, *e.g.*, using a visible spectra spectrophotometer. Alternatively, the label may be a chemilluminiscent one. The use of such labels is described in U.S. Patent Nos. 5,310,687, 5,238,808 and 5,221,605.

Further embodiments relate to isolated nucleic acid sequences encoding the polypeptides of this invention, in particular the nucleic acid sequences as set forth in SEQ. ID NO.4, 5, and 6, or being substantially similar to all or a portion thereof. The term substantially similar means having a least 50-70%, more preferably 70-80%, and most preferably 80 or 90% identity to one of said sequences. Such substantially similar nucleic acid sequences hybridize under high stringency southern hybridization conditions with at least one of the nucleic acid sequences set forth in SEQ ID Nos. 4, 5 or 6.

The nucleic acid molecule may be RNA or DNA, single stranded or double stranded, in linear or covalently closed circular form. For the purposes of defining high stringency southern hybridization conditions, reference can conveniently be made to Sambrook et al., 1989, Book 2, pp 9.31-9.58.

It will be appreciated that the sequence of nucleotides of this aspect of the invention may be obtained from natural, synthetic or semi-synthetic sources; furthermore, this nucleotide sequence may be a naturally occurring sequence, or it may be related by mutation, including single or multiple base substitutions, deletions, insertions and inversions, to such a naturally occurring sequence, provided always that the nucleic acid molecule comprising such a sequence is capable of being expressed as a *Helicobacter* antigen as broadly described above.

The nucleotide sequence may have expression control sequences positioned adjacent to it, such control sequences usually being derived from a heterologous source.

This invention also provides a recombinant DNA molecule comprising an expression control sequence having promoter sequences and initiator sequences and a nucleotide sequence which codes for a *Helicobacter* antigen, the nucleotide sequence being located 3' to the promoter and initiator sequences. In yet another aspect, the invention provides a recombinant DNA cloning vehicle capable of expressing a *Helicobacter* antigen comprising an expression control sequence having promoter sequences and initiator sequences, and a nucleotide sequence which codes for a *Helicobacter* antigen, the nucleotide sequence being located 3' to the promoter and initiator sequences. Cloning vehicles can be any plasmid (or vector) known in the art, including viral vectors, such as alphavirus pox viruses. In a further aspect, there is provided a host cell containing a recombinant DNA cloning vehicle and/or a recombinant DNA molecule as described above.

Suitable expression control sequences and host cell/cloning vehicle combinations are well known in the art, and are described by way of example, in Sambrook et al. (1989). One embodiment of this invention relates to expression systems that employ the use of plasmids. Preferred embodiments of the invention employ plasmids which exhibit high copy number upon replication in a transformed host cell. Copy number refers to number of copies of the plasmid, or the genes contained therein, which replicate in a host cell upon induction of the plasmid. The copy number within a cell determines the copies of the desired nucleotide sequence (or gene). This copy number equates to the gene dosage. Certain plasmids are characterized as high copy number. Generally, high copy number refers to a plasmid which generates at least about 100 copies per cell and preferably generating from about 100 to about 700 or 1,000 copies per cell. The selection of the appropriate copy number for the expression of the nucleotides sequences of this invention when combined with the selection of a strong promoter improves the amount of desired polypeptide that is generated in a host cell. In each high copy number plasmid, the nucleic acid sequence encoding a selected polypeptide is operably linked to a strong promoter (Hannig et al.). Plasmid copy number is determined by the nature the origin of replication of the plasmid and corresponding cis acting control elements, together these genetic elements are defined as a replicon. In plasmids which normally reside in *E. coli*, there are several different

replicons. Most common is pMB1 and its close relative, ColE1. Plasmids which carry these replicons maintain about 15-20 copies per cell under normal growth conditions. A small protein encoded by the *rop* gene adjacent to the origin of replication negatively regulates pMB1/ColE1 plasmid replication. Plasmids with deletions in the *rop* gene or mutations in the site of *rop* action have increased replication and higher copy number. One example of this is in the pUC plasmids whose copy number is increased to 500-700 copies per cell. Other lower copy number replicons are p15A (10-12 copies/cell), and pSC101 (5-10 copies per cell). Also, the moderate copy number of pMB1 and ColE1 plasmids can be modified to high copy numbers by inhibiting the *E. coli* protein synthesis.

It is further noted that the copy number exhibited in a specific host is the proper measurement of plasmid copy number. Plasmids which normally do not reside in gram negative hosts or *E. coli* hosts may not be regulated for replication when introduced into *E. coli* hosts. When such promoters are not regulated, they can exhibit high copy number in the selected host. An example of this is pNG2, a plasmid from the gram positive bacteria *Corynebacterium diphtheriae*. Whereas, the normal copy number for this plasmid in *C. diphtheriae* is 1-2 copies per cell, transformants of the plasmid in *E. coli* are estimated to have >100 copies of the plasmid (Serwold-Davis, T. et al., PNAS, 84: 4964-8, 1987).

Strong promoters are selected such they are easily regulated in order that they may be repressed during culture growth "towards maximal cell numbers. In many embodiments, the strong promoters can also be induced so that the host cell overproduces the recombinant polypeptide at a desired level, usually in excess of about 10 to about 30% total cellular protein. The promoter is selected to maintain the desired level of polypeptide expression. Exemplary promoters are T7 promoter from T7 bacteriophage, arabinose promoter for the *araBAD* operon, lambda phage promoters (such as  $P_L$  and  $P_R$ ) the *trc* and *tac* promoters. The T7 promoter is controlled by the T7 RNA polymerase gene which is a very active enzyme; it elongates RNA chains five times faster than the *E. coli* RNA polymerase. In addition the polymerase is very selective for specific promoter sequences and termination signals so that the action of the enzyme is targeted specifically to the gene of interest. In one system developed by

Studier et al. (referenced, infra), the T7 polymerase gene has been integrated into the host chromosome under the control of the lac promoter. Upon induction with IPTG, T7 RNA polymerase is produced and acts to transcribe a T7 promoter gene housed on an expression plasmid in the cell. In addition, a second plasmid expressing T7 lysozyme is included to control the background expression of the T7 polymerase. For T7, an alternative induction system for the expression of genes by the T7 promoter is to grow *E. coli* cells containing the T7 recombinant plasmid to the desired density and then introduce the T7 polymerase gene by infecting with a bacteriophage which carries that gene.

10

Expression can also be enhanced based on the choice of cellular compartmentalization. Outer membrane proteins are often difficult to overexpress recombinantly because of the requirement for transport to the outer membrane and correct insertion into that membrane. Overexpression of full length outer membrane proteins which contain the leader sequences will many times overcome the host cell export machinery leading to cessation of growth and low recombinant protein yield. Cloning the mature sequences of an outer membrane protein behind translation start signals provided by the expression vector can eliminate the need for the host cell to transport the protein to the membrane and allow the cell to overexpress the recombinant protein as inclusion bodies in the cytosol which are relatively stable and resistant to proteolysis.

20

One or more of the above considerations are included when selecting a desired host. In general, one can select or optimize the host based on factors which can influence the yield of recombinantly expressed proteins. These factors include growth and induction conditions, mRNA stability, codon usage, translational efficiency and the presence of transcriptional terminators to minimize promoter read through. For example, one can modify the ability of the host to produce proteases. Stress induced proteases in the host cell are induced during induction of recombinant protein expression, therefore, the use of protease deficient hosts and/or coexpression of chaperones can be employed to minimize the proteolysis of the recombinant upon induction of the protein in culture. In the case at hand, the polypeptides of the invention contain cysteine bonds. Accordingly, one can aid cysteine bond formation, for example

30

through the use of *trx* hosts, which can help with the proper folding in the cytosol by altering the redox potential in the cytosol.

Suitable host cell or host strains for the practice of this invention are *omp T lon*  
 5 host strains such as BL21, BLR, B834; *trx*B host strains such as AD494, *lon clpA*  
 mutant strains such as KY 42263 and *lon clpA hslVU* mutant strains such as KY 2266  
 (for the latter two strains the Kanemori, M. et al. J. Bact. 1997. 79:7219-7225).

When host cells, which are modified or selected for enhanced expression, are  
 10 combined with the high copy number plasmids (also referred to as over- expressing  
 plasmids) of this invention, the polypeptide is expressed as inclusion bodies. Preferably,  
 the nucleotide sequence selected for forming inclusion bodies is the nucleotide sequence  
 corresponding to the mature portion of the polypeptide. The signal sequence of the  
 desired polypeptide is not included in this nucleotide sequence for the mature  
 15 polypeptide. A desired *H. pylori* protein can be obtained by a method comprising: (a)  
 transforming a selected host cell with at least one high copy number plasmid, which  
 comprises the nucleotide sequence of interest operably linked to a strong promoter, and  
 (b) growing the transformed host cell in culture media. Often, it is advantageous to use  
 an inducible promoter to control the timing of the expression of the nucleotide sequence.  
 20 Basically, when the promoter is inducible, the rate of transcription increases in response  
 to the inducing agent or inducing conditions for the promoter. A selectable marker can  
 be used in the plasmid in order to grow the transformed host in the presence of a selecting  
 agent that works in combination with the chosen marker. The *H. pylori* polypeptide  
 produced by the above method is expressed as inclusion bodies and is also soluble in  
 25 detergent extractions, without the requiring denaturants, such as urea or guanidine for  
 solubilizing the inclusion bodies. These two characteristics are important since they  
 allow for the application of straightforward and practical means in isolating and  
 purifying the polypeptide. In the absence of expressing the polypeptide as an inclusion  
 body and as a soluble material, the difficulties in the purification steps are generally  
 30 overwhelming.



In yet further aspects, there are provided fused polypeptides comprising a *Helicobacter* polypeptide of this invention and an additional polypeptide, for example a polypeptide coded for by the DNA of a cloning vehicle, fused thereto. Such a fused  
5 polypeptide can be produced by a host cell transformed or infected with a recombinant DNA cloning vehicle as described above and it can be subsequently isolated from the host cell to provide the fused polypeptide substantially free of other host cell proteins.

Based on the above-identified specific sequences, one may obtain numerous  
10 additional isolated nucleic acid sequences encoding the polypeptides of this invention due to the degeneracy of the genetic code. Amino acids and their codons are well-known. Accordingly, using site-directed mutagenesis of one polypeptide of *H. pylori*, one can generate additional nucleic acid sequences, as desired. These methods of generating nucleic acid sequences and fragments thereof provide a convenient manner in  
15 which to generate portions of the polypeptides for fusion molecules.

Using the nucleic acid sequence described herein, one can generate synthetic polypeptides displaying the antigenicity of a *Helicobacter* isolated polypeptide of this invention. As used herein, the term "synthetic" means that the polypeptides have been  
20 produced by chemical or biological means, such as by means of chemical synthesis or by recombinant DNA techniques leading to biological synthesis. Such polypeptides can, of course, be obtained by cleavage of a fused polypeptide as described above and separation of the desired polypeptide from the additional polypeptide coded for by the DNA of the cloning vehicle by methods well known in the art. Alternatively, once the  
25 amino acid sequence of the desired polypeptide has been established, for example, by determination of the nucleotide sequence coding for the desired polypeptide, the polypeptide may be produced synthetically, for example by the well known Merrifield solid-phase synthesis procedure.

30 Once recombinant DNA cloning vehicles and/or host cells expressing a desired *Helicobacter* polypeptide of this invention have been constructed by transforming or transfecting such cloning vehicles or host cells with plasmids containing the corresponding *Helicobacter* nucleic acid sequence, cloning vehicles or host cells are

cultured under conditions such that the polypeptides are expressed. The polypeptide is then isolated substantially free of contaminating host cell components by techniques well known to those skilled in the art. In a preferred embodiment for purifying the desired polypeptide one can first follow the standard techniques of lysing the host cells  
5 and then isolating the inclusion bodies by removing soluble proteins and other contaminants potentially. In a second step, it is preferred to solubilize the inclusion bodies in a zwitterionic detergent, which are well-known in the art. The detergent may be used with a denaturant; however, surprisingly, no denaturant is required. Finally, the solubilized inclusion body material, is purified using cationic exchange gel  
10 chromatography, followed by eluting with a salt solution to collect the purified polypeptide. This method generates a polypeptide which is at least about 75 or 80% pure, preferably at least about 90%.

This invention also provides for a method of diagnosing an *H. pylori* infection  
15 comprising the step of determining the presence, in a sample, of an amino acid sequence EDDGFYTSVG YQIG EAAQMV (SEQ ID No.: 7), or preferably any of the isolated *H. pylori* polypeptides of this invention. Any conventional diagnostic method may be used. These diagnostic methods can easily be based on the presence of an amino acid sequence or polypeptide. Preferably, such a diagnostic method matches for a  
20 polypeptide having at least 10, and preferably at least 20, amino acids which are common to the polypeptides of this invention.

As noted, the present invention also relates to nucleic acid sequences encoding *H. pylori* polypeptides. The nucleic acid sequences disclosed herein can also be used for  
25 a variety of diagnostic applications. These nucleic acids sequences can be used to prepare relatively short DNA and RNA sequences that have the ability to specifically hybridize to the nucleic acid sequences encoding the polypeptides of this invention. Nucleic acid probes are selected for the desired length in view of the selected parameters of specificity of the diagnostic assay. The probes can be used in diagnostic  
30 assays for detecting the presence of pathogenic organisms in a given sample. With current advanced technologies for recombinant expression, nucleic acid sequences can be inserted into an expression construct for the purpose of screening the corresponding

oligopeptides and polypeptides for reactivity with existing antibodies or for the ability to generate diagnostic or therapeutic reagents.

In preferred embodiments, the nucleic acid sequences employed for hybridization studies or assays include sequences that are complementary to a nucleotide stretch of at least about 10 to about 20 nucleotides, although at least about 10 to 30, or about 30 to 60 nucleotides can be used. Nucleotide stretches of at least 10 nucleotides are beneficial for providing stability and selectivity when testing a clinical sample for *Helicobacter* infection. A variety of known hybridization techniques and systems can be employed for practice of the hybridization aspects of this invention, including diagnostic assays such as those described in Falkow et al., US Patent 4,358,535. Depending on the application, one will desire to employ varying conditions of hybridization to achieve varying degrees of selectivity of the probe toward a target sequence. For applications requiring a high degree of selectivity, one will select relatively low salt and/or high temperature conditions, such as provided by 0.02M-0.15M NaCl at temperature of about 50°C to 70°C. These conditions are particularly selective, and tolerate little, if any, mismatch between the probe and the template or target strand.

For some applications, if one desires to prepare mutants employing a mutant primer strand hybridized to an underlying template, less stringent hybridization conditions are called for in order to allow formation of the heteroduplex. The conditions may be altered by using 0.15M-0.9M salt, at temperatures ranging from about 20°C to about 55°C. In general, it is appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased temperature. Thus, hybridization conditions can be readily manipulated, and the method of choice will generally depend on the desired results.

In certain embodiments, one may desire to employ nucleic acid probes to isolate variants from clone banks containing mutated clones. In particular embodiments, mutant clone colonies growing on solid media which contain variants of an *H. pylori* polypeptide sequence could be identified on duplicate filters using hybridization

conditions and methods, such as those used in colony blot assays, to obtain hybridization only between probes containing sequence variants and nucleic acid sequence variants contained in specific colonies. In this manner, small hybridization probes containing short variant sequences of the *H. pylori* genes of the invention may be  
5 utilized to identify those clones growing on solid media which contain sequence variants of the entire genes encoding polypeptides of 75, 77 and 79 kDa as discussed herein. These clones can then be grown to obtain desired quantities of the variant nucleic acid sequences or the corresponding antigen.

10 In clinical diagnostic embodiments, nucleic acid sequences of the present invention are used in combination with an appropriate means, such as a label, for determining hybridization. A wide variety of appropriate indicator means are known in the art, including radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of giving a detectable signal. In preferred diagnostic embodiments, one will  
15 likely desire to employ an enzyme tag such as urease, alkaline phosphatase or peroxidase, instead of radioactive or other environmental undesirable reagents. In the case of enzyme tags, colorimetric indicator substrates are known which can be employed to provide a means visible to the human eye or spectrophotometrically, to identify specific hybridization with pathogen nucleic acid-containing samples.

20

In general, it is envisioned that the hybridization probes described herein will be useful both as reagents in solution hybridizations as well as in embodiments employing a solid phase. In embodiments involving a solid phase, the test DNA (or RNA) from suspected clinical samples, such as exudates, body fluids (*e.g.*, amniotic fluid, middle  
25 ear effusion, bronchoalveolar lavage fluid) or even tissues, is absorbed or otherwise affixed to a selected matrix or surface. This fixed, single-stranded nucleic acid is then subjected to specific hybridization with selected probes under desired conditions. The selected conditions will depend on the particular circumstances based on the particular criteria required (depending, for example, on the G+C contents, type of target nucleic  
30 acid, source of nucleic acid, size of hybridization probe, *et.*). Following washing of the hybridized surface so as to remove nonspecifically bound probe molecules, specific hybridization is detected, or even quantified, by means of the label.

The nucleic acid sequences which encode for the *H. pylori* polypeptides of the invention, or their variants, may be useful in conjunction with PCR<sup>TM</sup> technology as set out, e.g., in U.S. Patent 4,603,102, one may utilize various portions of any of *H. pylori* sequences of this invention as oligonucleotide probes for the PCR<sup>TM</sup> amplification of a defined portion of an *H. pylori* (75, 77 kDa) sequence may then be detected by hybridization with a hybridization probe containing a complementary sequence. In this manner, extremely small concentrations of *H. pylori* nucleic acid may be detected in a sample utilizing the nucleic acid sequences of this invention.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those skilled in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in the light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

### EXAMPLES

#### **Example 1**

**1.1 Bacterial strains.** *H. pylori* strains PBCC 1101, PBCC 1102, PBCC 1103, PBCC 1105, and PBCC 1107 were isolated from human gastric biopsies obtained from the University of Rochester School of Medicine and Dentistry (Rochester, NY). *H. pylori* strains LET 13 and RSD 14 were isolated from human gastric biopsies obtained from the Syracuse Veterans Administration Medical Center (Syracuse, NY). *H. pylori* strains MH 60 EG 52, RJ 17, LJ 63, and MJ 34 were obtained as frozen stocks from the Clement J. Zablocki VA Medical Center (Milwaukee, WI). *H. pylori* strain SS1 was originally isolated from a human gastric biopsy, and subsequently adapted to infect mice (obtained from A. Lee, University of New South Wales, Sydney, Australia). *H. pylori* strains obtained from American Type Culture Collection were ATCC 43504 and ATCC 43579. The *H. felis* strain was obtained from T. Blanchard, Case Western Reserve University, Cleveland, OH.

WO 00/00614

PCT/US99/14375

**1.2 Culturing of Helicobacter strains.** Cultures of *H. pylori* and *H. felis* were grown at 37°C on Columbia broth agar plates with 10% defibrinated horse blood and 10 µg/ml vancomycin in a microaerophilic chamber. Liquid cultures of *H. pylori* strains

5 were grown at 37°C in BHI medium with 4% fetal calf serum and 10 µg/ml vancomycin in flasks infused with a gas mixture of 10% CO<sub>2</sub>/ 6% O<sub>2</sub>/ 84% N<sub>2</sub> (vol/vol/vol).

**1.3 Preparation of *H. pylori* PBCC 1103.** Strain PBCC 1103 was grown as above.

10 The cell pellet was washed twice in a phosphate buffered saline (PBS) solution, and resuspended in a 0.3% formaldehyde solution for 1 hr. The fixed cells were then washed in and resuspended in PBS to an O.D.<sub>600</sub> of 0.1 (approximately 10<sup>7</sup> cfu).

**1.4 Injection of Mice with *H. pylori* PBCC 1103.** Ten 6 to 8 wk old female

15 BALB/c mice were primed by interperitoneal injection with ca. 10<sup>7</sup> formalin fixed PBCC 1103 cells at weeks 0, and boosted at weeks 2, 4 and 8. After a 34 week rest period a pre-fusion boost of approximately 10<sup>7</sup> formalin fixed PBCC 1103 was given interperitoneally at week 42.

20 **1.5 Production of Monoclonal Antibodies, Hybridoma Techniques and Screening Procedures.** During immunization and the rest period, mouse sera were obtained (week 6, 10) and tested for antibody activity by ELISA by using air-dried, formalin fixed PBCC 1103 cells, 0.1 ml per well at an absorbance of 0.100 (A<sub>620</sub>) as the coating antigen.

25

Spleens were recovered from five immunized mice about 72 hours after the last injection, and were combined with nonsecreting X63Ag8.653 mouse (BALB/c) myeloma cells in 5:1 ratio (splenocytes:myeloma). The cells were fused for four minutes in 50% (v/v) polyethylene glycol 1500 and 10% dimethylsulfoxide in

30 Dulbecco's Modified Eagle medium (D-MEM). The fused cells were diluted in selection medium, D-MEM supplemented with hypoxanthine, aminopterin, thymidine, 10% fetal bovine serum and 10% NCTC-109 media supplement (Gibco-BRL). The

WO 00/00614

PCT/US99/14375

fusion efficiency (wells with colony growth vs. number of wells seeded) was 100% (900/900).

Primary screening was completed by ELISAs using PBCC 1103 whole cell antigen as described above, partially purified protein mixture with urease and heat shock protein(s) and lipopolysaccharide (LPS) purified from PBCC 1103 cells. Secondary selection was completed by SDS-PAGE Western blot using heterologous ATCC 43579 whole cell lysate as antigen. Positive reactors were identified and designated 1 through 90, coded as hybridoma (Hpy) and saved for further characterization.

10

Selected hybridomas of interest were subcloned once by limiting dilution procedure. Monoclonal antibodies were provided as tissue culture supernatant (TCS), concentrated by 50% saturated ammonium sulfate precipitation (SAS-TCS) or ascites.

#### 15    **1.6    SDS-PAGE and Western Blot Analysis.**

SDS-PAGE was carried out as described by Laemmli using 10-18% (w/v) acrylamide gels (Zaxis, Hudson, OH). Proteins were visualized by staining the gels with Coomassie ProBlue (Owl Separation Systems, Woburn, MA). Gels were scanned using a Personal Densitometer SI (Molecular Dynamics Inc., Sunnyvale, CA) and molecular weights estimated using the Fragment Analysis software (version 1.1) and prestained molecular weight markers obtained from Owl Separation Systems. Transfer of proteins to polyvinylidene difluoride (PVDF) membranes was accomplished using a semi-dry electroblotter and electroblot buffers (Owl Separation Systems). Membranes were probed with the indicated antisera followed by goat anti-mouse or anti-rabbit alkaline phosphatase conjugate as the secondary antibody (BioSource International, Camarillo, CA). Western blots were developed using the BCIP/NBT Phosphatase Substrate System from Kirkegaard and Perry Laboratories (Gaithersburg, MD).

#### 30    **1.7    Conservation of the 75 and 77 kDa Proteins in *H. pylori* Strains.**

Tissue culture supernatants from hybridomas were analyzed by whole cell ELISA

- and antigen ELISA, followed by Western analysis using whole cell lysates of a number of *H. pylori* strains. Parent hybridoma designated **Hpy 64** was determined by these methods to react to proteins in the range of approximately 75-79 kDa. Subsequent cloning produced the monoclonal antibody (MAb)
- 5 designated **Hpy 64-27**, which has reactive epitopes in 10 of 12 *H. pylori* strains tested, but not in *H. felis* (Table 1).



**Table 1**Reactivity of MAb Hpy64-27 with Heterologous *H. pylori*Strains and an *H. felis* strain (Conservation of 75/77 kDa Proteins)

| <u>Strain</u> | <u>Reactivity</u> | <u>Strain</u>      | <u>Reactivity</u> |
|---------------|-------------------|--------------------|-------------------|
| ATCC 43579    | ++                | EG52               | ++                |
| PBCC 1101     | ++                | RJ17               | -                 |
| PBCC 1102     | +/-               | LJ63               | +/-               |
| PBCC 1103*    | ++                | MJ34               | +                 |
| PBCC 1105     | ++                | PBCC 1107          | ++                |
| MH60          | ++                | <i>H. felis</i> ** | -                 |
| SS1           | -                 |                    |                   |

5

\* -homologous isolate

\*\* non-*pylori*

++ : strongly reactive

+ : reactive

+/- : slightly reactive

- : no detectable reactivity

- 10 **1.8 Purification of the 75 and 77 kDa Proteins.** Bacterial cells (ca. 10 g wet wt of *H. pylori* ATCC 43579) were resuspended in 70 ml of 0.05 M HEPES / 10 mM EDTA / 1.0 mM PMSF (pH 7.0) by homogenization using a Tekmar Ultra-Turrex tissue homogenizer. The cells were disrupted by sonication using a Branson Sonifier Cell Disrupter. The disrupted cells, including the membrane
- 15 fraction, were pelleted by centrifugation at 42,000 rpm using a Beckman 70Ti rotor for 40 min at 4°C. Following centrifugation, the pellet was resuspended in 70 ml of 0.01 M HEPES / 1.0 mM MgCl<sub>2</sub> / 1.0 mM PMSF / 1.0% TX-100 (pH 7.4) and stirred for 1 hr at room temp. The suspension was centrifuged at 42,000 rpm using a Beckman 70Ti rotor for 40 min at 4°C. Following centrifugation, the
- 20 pellet was resuspended in 70 ml of 0.05 M Tris-HCl / 10.0 mM EDTA / 1.0% ZWITTERGENT™3-14 (pH 7.4) and stirred for 1 hr at room temp. The suspension was then centrifuged at 42,000 rpm using a Beckman 70Ti rotor for 40 min at 4°C. Following centrifugation, the supernatant containing the 75 and 77 kDa proteins was collected and stored at -20°C for further purification.

The ZWITTERGENT™ 3-14 crude extract was buffer exchanged by passage over a 250 ml Sephadex G-25 (coarse) column (Pharmacia) equilibrated in 0.02 M Tris-HCl / 5.0 mM EDTA / 1.0% ZWITTERGENT™ 3-14 (pH 8.0). Approximately one half (35 ml) of the buffer exchanged ZWITTERGENT™ 3-14 crude extract preparation was loaded onto a 10 ml Pharmacia SP Fast Flow Sepharose column equilibrated 0.02 M Tris-HCl / 5.0 mM EDTA / 1.0% ZWITTERGENT™ 3-14 (pH 8.0). Unbound protein was washed through the column with an additional 2 bed volumes of equilibration buffer. The 75 and 77 kDa proteins were co-eluted using a linear NaCl gradient (0-15 M NaCl) in 0.02 M Tris-HCl / 5.0 mM EDTA / 1.0% ZWITTERGENT™ 3-14 (pH 8.0). Fractions were screened for the 75 and 77 kDa proteins by SDS-PAGE / Western and pooled. Twelve ml of pooled co-eluting 75 and 77 kDa was subsequently applied to a 500 ml Pharmacia Superose-12 column equilibrated in phosphate buffered saline (PBS) / 1.0% ZWITTERGENT™ 3-14. Fractions were screened for the co-eluting 75 and 77 kDa proteins by SDS-PAGE / Western and pooled. Material from two independent SP Sepharose / Superose-12 runs was combined and the co-eluted protein was precipitated by the addition of 9 volumes of ethanol overnight at -20°C. The resulting suspension was then centrifuged at 9,000 rpm using a Beckman SS31 rotor for 30 min at 4°C and the pellet was resuspended in 10 ml of PBS / 1.0% ZWITTERGENT™ 3-14. Purified 75 and 77 kDa proteins were stored at -20°C. The protein isolated using this protocol exhibited two bands on SDS-PAGE corresponding to subunit molecular masses of 75 kDa and 77 kDa (Figure 1A).

**Protein Estimation.** Protein concentration was estimated by the BCA assay from Pierce (Rockford, IL) using BSA as a standard.

**1.9 Reactivity of MAb 64-27 clone with 75 kDa and 77 kDa.** Western blot analysis of co-purified 75 kDa and 77 kDa proteins revealed that only the 75kDa protein reacts strongly with the MAb 64-27 (Figure 1B).

## **Example 2**

**2.1 Production in Mice of Polyclonal Antisera to Purified 75 kDa and 77 kDa Proteins.** Two groups of 10 Swiss Webster mice were used for production of

polyclonal antisera to the purified 75 kDa and 77 kDa proteins. One group was injected with 25 µg of the purified protein with incomplete Freund's adjuvant (IFA) on weeks 0 and 4. The other group was injected with 25 µg of the purified proteins with 50 µg of MPL™ on weeks 0 and 4. Both groups were bled on week 6.

5

**2.2 Surface Labeling by Immunoelectron Microscopy (IEM).** Twenty microliter droplets of live PBCC 1105 in broth were placed on parafilm. Three Hundred mesh gold carbon-coated formvar grids were placed film side down on the droplets. Grids with cells were then rinsed in droplets of PBS/BSA buffer and transferred to PBS containing 1% cold water fish gelatin. After blocking, grids were incubated in mouse polyclonal antisera diluted 1:50 in PBS/BSA for 1 hr, then rinsed in buffer. Grids were then incubated on a fifty-fold dilution of Nanogold (Nanoprobes, Inc., Stony Brook, NY), washed in buffer, and fixed with a solution of 1% glutaraldehyde in PBS. The fixative was removed from the grids with deionized water. The HQ silver enhancement kit (Nanoprobes, Inc.) was used to nucleate the Nanogold. The grids were then stained with Nanovan (Nanoprobes, Inc.) and viewed on a Zeiss 10C transmission electron microscope operating at 100 Kv. The polyclonal antiserum obtained from injecting mice with live strain PBCC 1103 was able to label the surface of heterologous strain PBCC 1105. As seen in Figure 2, the 75/77 kDa proteins are surface localized and that the reactive epitopes are present in both *H. pylori* strains.

**2.3 Flow Cytometry.** Approximately  $10^6$ - $10^7$  cells of PBCC 1105 (O.D.<sub>600</sub> range of 0.2 to 1.6) were washed once in PCM buffer (10 mM NaPO<sub>4</sub>, pH 7.4, 150 mM NaCl, 0.5 mM MgCl<sub>2</sub>, 0.15 mM CaCl<sub>2</sub>). The cell pellet was gently resuspended in 100 ml PCM buffer. One microliter of polyclonal antisera was added and the cells incubated at 37 °C for 30 min on a rocker. Cells were diluted with 900 µl PCM buffer, mixed gently and pelleted at maximum speed in a microcentrifuge for 1 min. The pellet was gently resuspended in 100 µl of PCM buffer. One microliter of Oregon Green 514 conjugated goat anti-mouse antibody (Molecular Probes) was used to fluorescently label the cells tagged with the polyclonal antisera at 37°C for 30 min. The cells were then washed as described above and diluted in 1 ml PCM for analysis on the FACSsort (Becton-Dickenson). Sera obtained from mice prior to injection with the mixture of the 75 kDa

and 77 kDa proteins on day 0 showed only background labeling in all five strains tested, with a mean fluorescent intensity of less than 10 (see Figure 3A). Polyclonal antisera obtained from the same mice on day 49 following two injections of the protein mixture displayed a positive fluorescent peak with all strains tested (strains ATCC 43579, PBCC 1105, ATCC 43504 and SS-1: See Figure 3B). The mean fluorescent intensity ranged from 36.44 for SS1 to 322.68 from the homologous strain ATCC 43579. This demonstrates that there are conserved epitopes among these strains. Without being bound by theory, the difference in the fluorescent intensities could be a function of the number of antigen molecules expressed on the cell surface or the antigenic variability of the epitopes among the heterologous strains.

### Example 3

**3.1 Assay of Bactericidal (BC) Activity of Polyclonal Antisera.** Target cells of *H. pylori* strain PBCC 1105 for the BC assay were provided on the morning of the assay. The optical density of the liquid culture at 600 nm was approximately 0.1. Cells pelleted from 10 ml of this liquid culture were used to adsorb any nonspecific bactericidal activity in the complement source (human complement). Cells and serum were allowed to react for at least 1 hour on ice with occasional agitation. After incubation, the serum and cell mixture was centrifuged, and the serum was removed and placed on ice until needed. The BC assay was performed when the liquid culture attained an O.D.<sub>600</sub> of approximately 0.3. These cells were diluted in PCM buffer to a concentration of  $10^6$  cfu per ml. The reaction consisted of 10  $\mu$ l strain PBCC 1105 at a concentration of  $10^6$  cfu per ml, 10  $\mu$ l adsorbed serum as a complement source, 5  $\mu$ l of diluted polyclonal antisera (heat inactivated 60°C for 10 min), and 25  $\mu$ l of PCM. The reaction was incubated at 36°C in a 10% CO<sub>2</sub> chamber for 30 min. Two hundred  $\mu$ l of PCM was then added to the reaction and duplicate 50  $\mu$ l aliquots were plated on Columbia agar with 10% defibrinated horse blood and 10  $\mu$ g/ml vancomycin. Plates were incubated in a microaerophilic chamber for at least 72 hours, and counted on an automated plate reader. Polyclonal antisera from mice injected with the 75/77 kDa protein mixed with Incomplete Freund's Adjuvant (IFA) had a BC<sub>50</sub> at a dilution of greater than 1/3200 and 75/77 kDa protein mixed with MPL™ had a BC<sub>50</sub> at a dilution of greater than 1/3200, as compared to 1/800 in serum from control mice (see Figure 4,

complete data for MPL is not shown). This is another indication that the epitopes of the 75/77 kDa proteins are surface exposed.

#### Example 4 - Immunization and Challenge Tests

- 5    **4.1    Immunizations.** C57Bl/6 mice were vaccinated intragastrically on days 0, 2, 14, and 16 with 100 µg recombinant *H. pylori* (Hp) urease (rUre) or native Hp 75/77 kDa protein formulated with 10 µg cholera toxin (CT); or were vaccinated subcutaneously on days 0 and 16 with 10 µg recombinant Hp rUre or native Hp 75/77 kDa protein formulated with 100 µg Aluminum Phosphate (AlPO<sub>4</sub>). Control mice
- 10    received no vaccine.
- 4.2    Reduction of Colonization in the Mouse Model.** Infection was established by the intragastric deposition of one 0.1 ml inoculum of *H. pylori* strain SS1 on day 37. The challenge inocula contained a suspension of *H. pylori* at a concentration of  $2 \times 10^8$
- 15    cfu per ml. The amount of viable *H. pylori* in the stomachs of mice was determined 2, 4 and 8 weeks following challenge (days 50 and 64). At each time point, 5 mice were sacrificed by cervical dislocation, their stomachs harvested and split into 2 longitudinal sections. One section from each mouse was homogenized and the homogenate diluted and plated on Columbia agar containing defibrinated horse serum and the appropriate
- 20    antibiotics. Plates were incubated at 37°C in a microaerophilic incubator for 5 days. After incubation the number of viable *H. pylori* isolated were enumerated. Total cfu per gram of stomach tissue were obtained by multiplying colony numbers per plate by the dilution factor and dividing by the weight of the sectioned stomach; numbers were transformed and are expressed as log<sub>10</sub> cfu per gram homogenized stomach tissue  $\pm$  1
- 25    standard error of the mean. Mice which were vaccinated intragastrically with the 75/77 kDa protein mixture admixed with CT showed a significant reduction in colonization following challenge with Hp strain SS1, as compared to non-vaccinated controls. At four weeks post-challenge, using either oral or parenteral routes of immunization, the number of colony forming units recovered per gram of stomach tissue was reduced by
- 30    approximately 1-2 logs in vaccinated mice (see Figure 5). This result is comparable to, if not improved over, the reduction that is afforded by vaccination with recombinant urease admixed with CT.

**Example 5 - Protein Analysis****5.1 Enzymatic Cleavage of the 75 kDa and 77 kDa Proteins.**

5 (i) **Trypsin cleavage.** Approximately 0.5 mg of the 75 kDa and 77 kDa protein mixture was precipitated with 90% (v/v) ethanol and the pellet resuspended in a total volume of 1 ml of phosphate-buffered saline (PBS) / 0.1% ZWITTERGENT™ 3-14. Ten microliters of a 0.5 mg/ml solution of trypsin was added (Boehringer Mannheim, Indianapolis, IN), and the reaction mixture incubated for 4 hr at 37°C.

10 (ii) **Elastase cleavage.** Approximately 0.5 mg of the 75 kDa and 77 kDa protein mixture was precipitated with 90% (v/v) ethanol and the pellet resuspended in a total volume of 1.0 ml of PBS / 0.1% ZWITTERGENT 3-14. Five microliters of a 1.0 mg/ml solution of elastase was added (Worthington), and the reaction mixture incubated for 4 hr at 37°C.

15 (iii) **Endoproteinase Lys-C cleavage.** Approximately 0.5 mg of the 75 kDa and 77 kDa protein mixture was precipitated with 90% (v/v) ethanol and the pellet resuspended in a total volume of 1.0 ml of PBS / 0.1% ZWITTERGENT 3-14. This preparation was added directly to a vial containing 5 mg of endoproteinase Lys-C  
20 (Boehringer Mannheim). The reaction mixture was incubated for 4 hr at 37°C.

(iv) **Separation of peptides.** The cleavage reaction mixtures from (i) -(iii) above were centrifuged in an Eppendorf centrifuge at 12,000 rpm for 5 min and the supernatant loaded directly onto a Vydac Protein C4 HPLC column (The Separations  
25 Group, Hesperia, CA). The solvent system used consisted of 0.1% (v/v) aqueous trifluoroacetic acid (TFA), (Solvent A) and acetonitrile : H<sub>2</sub>O : TFA, 80:20:0.1 (v/v/v) (Solvent B) at a flow rate of 1.0 ml/min. Following initial wash with Solvent A, the peptides were eluted with a linear gradient from 0-100% Solvent B in 30 min and detected by absorbance at 220 nm. Suitable fractions were collected, dried down in a  
30 Speed-Vac concentrator (Jouan Inc., Winchester, VA) and subsequently resuspended in distilled water. The fractions were subjected to SDS-PAGE using 10-18% (w/v, acrylamide) gradient gels (Owl Separation Systems) in a Tris-Tricine buffer system (Schögger and von Jagow). The fractions exhibiting a single peptide band were

WO 00/00614

PCT/US99/14375

submitted for N-terminal sequence analysis. Fractions displaying multiple peptide bands in SDS-PAGE were electrophoretically transferred onto a PVDF membrane as described above. The membrane was stained with Coomassie Brilliant Blue R-250 and individual bands excised and submitted for N-terminal sequence analysis (Matsudaira),  
5 as discussed in Example 6 below.

## 5.2 Estimation of Molecular Weight By MALDI-TOF Mass Spectrometry.

Determination of molecular weight by Matrix Assisted Laser Desorption / Ionization - Time of Flight (MALDI-TOF) mass spectrometry (Hillenkamp and Karas) was carried  
10 out using a Lasermat 2000 Mass Analyzer (Finnigan Mat, Hemel Hempstead, UK) with 3,5-dimethoxy-4-hydroxy-cinnamic acid as the matrix. For samples containing 1.0% ZWITTERGENT™ 3-14, cold ethanol precipitation was carried out twice to remove the detergent using a 90% (v/v) final ethanol concentration followed by solubilization of the precipitated protein in water.. MALDI-TOF analysis using 3,5-dimethoxy-4-hydroxy-  
15 cinnamic acid matrix in presence of 70% (v/v) aqueous acetonitrile / 0.1% TFA resulted in the identification of predominantly two species with average molecular weights of 75,572 kDa and 77,633 kDa.

## Examples 6 - N-terminal Sequence Analysis

20 **6.1 Amino Acid Sequence Analysis.** N-terminal sequence analysis was carried out using an Applied Biosystems Model 477A Protein/Peptide Sequencer equipped with an on-line Model 120A PTH Analyzer (Applied Biosystems, Foster City, CA). The phenylthiohydantoin (PTH) derivatives were identified by reversed-phase HPLC using a Brownlee PTH C-18 column (particle size 5 mm, 2.1 mm i.d. x 22 cm l.; Applied  
25 Biosystems).

**6.2 N-terminal Sequence Analysis of Both Intact 75 kDa and 77 kDa Proteins and Internal Peptides.** Determination of the N-terminal sequence of both 75 kDa and 77 kDa from intact proteins blotted onto PVDF exhibited identical sequence through the  
30 first 20 amino acids, EDDGFYTSVGYQIGEEAQMV (SEQ. ID No.: 7) of the mature processed form of these proteins, i.e. after cleavage of their signal peptides. This sequence was shown to match the N-terminus of three translated gene sequences identified from the *H. pylori* genome (Note: the third gene corresponds to a 79 kDa *H.*

## ART 34 AMDT

*pylori* genome (Note: the third gene corresponds to a 79 kDa *H. pylori* protein as noted in Example 7). In order to determine which of the three genes encode the 75 kDa and 77 kDa proteins, internal peptide fragments were generated from the mixture and subjected to N-terminal sequence analysis. Table 2 shows the N-terminal sequences obtained for both the intact proteins as well as fragments generated from the digestion of the mixture as described in Example 5. Sequence matches with the primary sequence deduced from the respective gene sequence are also indicated for each fragment. The data suggests that the mixture includes the 75 kDa and 77 kDa gene products because there is sequence identity specific to the respective genes. This result, in turn, is consistent with the mass results obtained by MALDI-TOF.

Table 2

## Fragment Sequence Summary

(positions are listed starting from the first amino acid of the leader sequence)

15

20

25

| Sequence              | 75 kDa  | 77 kDa  | 79 kDa  | SEQ ID No. |
|-----------------------|---------|---------|---------|------------|
| EDDGFYTSVGYQIGEEAQM   | 21-40   | 21-40   | 21-40   | 34         |
| EDDGFYTSVGYQIGEEAQMVK | 21-41   |         |         | 35         |
| STSTTIFNNEPGYR        |         | 135-149 |         | 36         |
| TGGKPN-P----WS        | 215-228 |         |         | 37         |
| TTTQTIDGK             |         | 226-234 |         | 38         |
| NSIAHFGTQE-QI         | 422-434 | 447-459 | 459-471 | 39         |
| VPNAQSLQNVVSK         | 468-481 | 493-506 | 505-517 | 40         |
| SKKNNPYSPQGIET        | 479-492 | 504-517 | 516-529 | 41         |
| NYYLNQN               | 493-499 | 518-524 | 530-536 | 42         |

**Example 7 - Identification of the 75 kDa, 77 kDa and 79 kDa Genes in the Chromosome of *H. pylori*.**

30 7.1 Genetic Methods. Isolation of plasmid and chromosomal DNA, agarose gel electrophoresis and restriction enzyme digestion were performed following standard



protocols (Sambrook et al.). DNA ligation was performed using a TOPO ligation kit (Invitrogen) following the recommended protocol.

7.2 **Oligonucleotide Synthesis.** Single stranded PCR primers were synthesized on  
5 an Applied Biosystems model 380B DNA synthesizer using  $\beta$ -cyanoethyl phosphoramidite chemistry.

7.3 **DNA Sequencing.** DNA sequencing was obtained by asymmetric PCR  
amplification using the fluorescent dye-labeled dideoxynucleotide terminator method  
10 (Gyllenstein et al., 1988). The dye-labeled single stranded DNA fragments were separated and identified with an Applied Biosystem model 373A automatic sequencing apparatus. Primary sequence information was analyzed using MacVector DNA analysis program (IBI, New Haven CT).

15 7.4 **PCR Amplification.** PCR amplifications were performed in 500  $\mu$ l tubes containing 100  $\mu$ l reaction volume overlaid with mineral oil. Each reaction contained 10 mM Tris-HCL, pH 8.3, 50 mM KCl and 1.5 mM  $MgCl_2$ ; 2.5 units Taq polymerase (Boehringer Mannheim), 200 mM dNTPs; 20 mM oligonucleotide primers and 1-2  $\mu$ g chromosomal DNA. Templates were amplified for 30 cycles with a 1 second extension  
20 on the annealing time of each cycle in a Perkin-Elmer Cetus thermocycler.

7.5 **Search Programs.** DNA alignment searches were performed using the MacVector program (IBI, New Haven CT). Protein homology searches were performed using either MacVector or DNASTAR (DNASTAR, Inc.) clustal alignment programs.

25 7.6 **Identification of Genome Homology to a Degenerate Oligonucleotide.** The *H. pylori* genome (unannotated) was downloaded from TIGR (The Institute for Genomic Research) ftp file and stored as a MacVector file. The N-terminal amino acid sequence of the 77 kDa *H. pylori* protein was entered into MacVector program and reverse  
30 translated to give the degenerate DNA oligonucleotide which was aligned to the genome. The oligonucleotide used to align to the *H. pylori* genome had the following sequence:

5'GARGAYGAYGGNTTTYTAYACNWSNGTNGGNTAYCARATHGGNGAR  
GCNGCNCARATGGTN 3' [SEQ. ID NO. 33].

Three matches were observed, corresponding to nucleotide positions 1404865-1404807, 722946-722888 and 352616-352558, all on the negative strand of the genome.

- 5 MacVector was used to outline open reading frames which would overlap the above regions in the genomic DNA. Three open reading frames based on translational start and stop codons were found. An open reading frame between nucleotides 1402991-1404925 (negative strand) predicted a protein of 81,157 kDa. There was a putative signal sequence of 20 amino acids which when cleaved would create a protein of 79 kDa
- 10 starting with the amino acids EDDGFYTSVGYQIGEEAQMV (SEQ ID No.7). A second open reading frame between nucleotides 720808-723006 (negative strand) predicted a protein of 79,123 kDa. There was a putative signal sequence of 20 amino acids which when cleaved would create a protein of 77 kDa starting with the amino acids EDDGFYTSVGYQIGEEAQMV (SEQ ID No.7). A third open reading frame
- 15 between nucleotides 350550-352672 (negative strand) predicted a protein of 77,687 kDa. There was a putative signal sequence of 19 amino acids which when cleaved would create a protein of 75 kDa starting with the amino acids EDDGFYTSVGYQIGEEAQMV (SEQ ID No.7).

- 20 The three proteins have identical 20 amino acids at the N-terminus and share internal peptide residues empirically determined from purified protein preparations (compare SEQ ID Nos. 1, 2, 3 and 7).

### Example 8

- 25 **8.1 PCR Amplification and Cloning of the 77 kDa Gene.** PCR primers were synthesized which hybridized to the translational start and stop codons of the ORF. The PCR primers synthesized to amplify the 77 kDa gene from the chromosome of *H. pylori* had the following sequences: Forward Primer: 5' GGC CAT ATG AAA AAA CAC ATC CTT TCA TTA GCT TTA GGC TCG 3' (SEQ ID No.: 8) and Reverse Primer: 5'
- 30 GGC AAG CTT GGG AGT TTC ACA AAA AGC TTA GTA AGC GAA CAC 3' (SEQ ID No.: 9). Chromosomal DNA from strains ATCC 43504, ATCC 43579, PBCC 1103, PBCC 1105, PBCC 1107, LET 13, RSD 14, and SS1 were used as template DNA. Conditions on the first round of PCR were 1 sec at 95°C, 1 sec at 60°C, 2 sec at 72°C,

WO 00/00614

PCT/US99/14375

with a 5 min hot start at 95°C (30 cycles). Ten microliters electrophoresed on an agarose gel revealed a 2 kb DNA fragment for strains ATCC 43504, PBCC 1103, PBCC 1107, and LET 13. The PCR was repeated under lower annealing temperatures (55°C) to amplify strains ATCC 43579 and PBCC 1105. Under these conditions,  
5 amplifications with these DNA templates were observed to have a diffuse band at 2 kb. A 2 kb family of bands was also observed after PCR amplification of SS1 DNA at 50°C annealing temperature.

Two microliters of each PCR amplification was added to a TOPO ligation mix  
10 (Invitrogen) and transformed into Top10F' competent cells. Ampicillin resistance colonies showing a white color on X-gal IPTG plates were picked and grown for mini plasmid preparations (three for each strain). A single transformant for each strain which was confirmed to have an insert of 2 kb was picked for large scale DNA preparation and sequence analysis.

15

Sequence analysis confirmed that the genes for the 77 kDa protein from strains ATCC 43504, ATCC 43579, PBCC 1103, PBCC 1105 and the 79 kDa protein from strains PBCC 1107, LET 13, and SS1 have been cloned successfully as described above.

20

### **Examples on Cloning, recombinant expression, and purification of the 75, 77, and 79 kDa proteins**

**Strains and growth conditions.** *E. coli* strain BLR(DE3) pLysS was obtained from Novogen. *E. coli* strains TOP10 and Top10F' were obtained from Invitrogen. *E. coli*  
25 strains were grown in HYSOY buffer containing 1% glucose or glycerol. Ampicillin (100 µg/mL) and chloramphenicol (30 µg/mL) were added when appropriate.

**DNA Manipulations.** Ligations into expression vectors were performed as described by Sambrook et al.

30

#### **Example 9**

##### **9.1 Cloning and expression of the 75 kDa gene from *H. pylori* strain ATCC43579.**

PCR amplification, cloning and sequencing of 70 kDa family genes from 7 strains of *H. pylori* revealed genes for the 77K kDa and the 79 kDa genes, but not the 75 kDa gene. Alignments of the three gene sequences published by TIGR revealed that the ATG start region of 75 kDa was different from that of the 77 kDa and 79 kDa genes and would not be expected to be amplified by the primer pair A and B (Table 3) used to amplify the 77 and 79 kDa genes. A primer corresponding to the 75 kDa gene start region could not be designed because a stretch of 11 CT dinucleotide repeats exists 10 bp downstream from the ATG start codon. CT dinucleotide repeats are also found in other *H. pylori* genes, and therefore a primer containing the repeats would not be specific for the 75 kDa gene.

DNA primers were designed based on the predicted sequences in and surrounding the 75 kDa gene sequence as published by TIGR. A primer was designed to anneal upstream from the 75 kDa coding region (Table 3, primer C). Attempts to PCR amplify the 75 kDa gene from strain ATCC 43579 using primers B and C under a variety of annealing conditions did not yield a PCR product. In order to obtain the 75 kDa gene from strain ATCC 43579, two complimentary internal primers were made based on empirically determined internal peptide sequence (Table 3, primers F and G). The front and back halves of the 75 kDa gene were amplified in separate PCR reactions using primer pairs D and G or E and F (Table 3), cloned and sequenced to verify that they were homologous to the predicted 75 kDa sequence.

Two additional primers (Table 3, H and I) were designed with BsmBI ends for seamless cloning to the two 75 kDa gene fragments. Upon PCR amplification of the front and back halves with primer pairs D and I or E and H, the resulting DNA fragments were digested with BsmBI and ligated to each other. The ligated DNA was amplified with primers D and E, corresponding to the ends of the mature open reading frame. A 2 kb DNA fragment was detected by ethidium bromide stained agarose gel electrophoresis. This DNA band was cloned in plasmid pCR2.1 (Invitrogen, San Diego, CA ) resulting in pPX7768 and sequenced to confirm that it was the 75 kDa gene (Figure 6, SEQ ID NO. 21). The predicted protein translation from this sequence is shown in Figure 7, SEQ ID NO 19.

The mature gene fragment was PCR amplified using primer J and K (Table 3) with pPX7768 as template DNA. The fragment was cloned into pRSETb at the

NdeI mECoRV sites to produce plasmid pPX7811. This plasmid was transformed into the expression strain BLR(DE3)pLysS, selecting for ampicillin and chloroamphenicol resistance. The strain was grown to mid-logarithmic phase in HYSOY broth (Difco) containing ampicillin and chloroamphenicol and expression was induced by the addition of 1mM isopropanol beta-D-thiogalactopyranoside (IPTG). Expression proceeded for 2 hours, after which the cells were harvested by centrifugation. Whole cell lysates were run on 10%SDS-PAGE. The 75kDa protein band was overexpressed as determined by coomassie stained gels and reacted with mAB Hpy 64-27 on Western blots

## 10 9.2 Purification of the recombinant 75 kDa protein

Bacterial cells (ca. 15 g wet wt of *E. coli* BLR(DE3)pLysS / pPX7811) were resuspended in 75 ml of 10 mM NaPO<sub>4</sub> / 150 mM NaCl / 5.0 mM EDTA / 1.0 mM Pefabloc (pH 7.2) by homogenization using a Ultra-Turrex tissue homogenizer (IKA Works, Wilmington, NC). The cells were disrupted by sonication using a Branson Sonifier Cell Disrupter. Inclusion bodies containing the recombinant 75 kDa protein were isolated by centrifugation at 10,000 rpm using a Sorvall SLA-1500 rotor for 30 min at 10°C. Following centrifugation, the pellet was resuspended by homogenization in 75 ml of 10 mM NaPO<sub>4</sub> / 150 mM NaCl / 5.0 mM EDTA / 4.0% TX-100 (pH 7.2) and stirred for 1.5 hr at 4°C. The suspension was centrifuged at 10,000 rpm using a Sorvall SS-34 rotor for 30 min at 10°C. Following centrifugation, the pellet was resuspended by homogenization in 75 ml of 10 mM NaPO<sub>4</sub> / 150 mM NaCl / 5.0 mM EDTA / 1.0% Zwittergent 3-14 (Z 3-14) (pH 7.2) and stirred for 1.5 hr at room temp. The suspension was centrifuged at 10,000 rpm using a Sorvall SS-34 rotor for 30 min at 10°C. Following centrifugation, the pellet was resuspended by homogenization in 75 ml of 10 mM NaPO<sub>4</sub> / 150 mM NaCl / 5.0 mM EDTA / 1.0% Zwittergent 3-16 (Z 3-16) (pH 7.2) and stirred for 2 hr at room temp. The suspension was centrifuged at 10,000 rpm using a Sorvall SS-34 rotor for 30 min at 10°C. Following centrifugation, the Z 3-14 and Z 3-16 supernatants containing the recombinant 75 kDa protein were collected and stored at -20°C for further purification. The Z 3-14 extract was buffer exchanged by passage over a 180 ml Sephadex G-25 (coarse) column (Pharmacia Biotech Inc., Piscataway, NJ) equilibrated in 0.02 M Tris-HCl / 5.0 mM EDTA / 1.0% Z 3-14 (pH 8.0). The buffer exchanged Z 3-14 crude extract preparation was loaded onto a 10 ml SP-Sepharose Fast Flow column (Pharmacia Biotech Inc.) equilibrated with 0.02 M Tris-HCl / 5.0 mM EDTA / 1.0% Z 3-14 (pH 8.0). Unbound protein was washed through the

WO 00/00614

PCT/US99/14375

column with an additional 2 column volumes of equilibration buffer. The recombinant 75 kDa protein was eluted using a linear NaCl gradient (0-0.5 M NaCl) in 0.02 M Tris-HCl / 5.0 mM EDTA / 1.0% Z 3-14 (pH 7.0) over 8 column volumes. Fractions were screened for recombinant 75 kDa protein by SDS-PAGE and Western analysis and pooled. Pooled  
 5 fractions were dialyzed into PBS / 1.0% Z 3-14 overnight at 4°C. The Z 3-16 extract was processed as described for the Z 3-14 extract.

### Example 10

#### 10.1 Cloning and expression of the 77 kDa gene from *H. pylori* strain ATCC 43579

10 Based on the published DNA sequence from TIGR, DNA oligonucleotide primers corresponding to the gene start (5') and end (3') were designed (Table 3, primers A and B). Chromosomal DNA from *H. pylori* strains ATCC 43579, ATCC 43504, PBCC 1103 and PBCC 1105 was isolated and used as templates for PCR amplification. Following PCR amplification, a 2 kb band was cloned into PCR2.1 TA cloning vector, and the *H.*  
 15 *pylori* insert was sequenced. DNA sequences of the 77 kDa genes from strains ATCC 43504, PBCC 1103 and PBCC 1105 were only partial but showed more homology to the 77 kDa gene from TIGR than to the 75 kDa or 79 kDa genes. The 77 kDa gene from strain ATCC 43579 was completely sequenced (plasmid pPX7760b). The DNA sequence of the complete coding region is shown in SEQ ID NO. 5. The predicted  
 20 protein translation from this sequence is shown in SEQ ID NO. 2.

The 77 kDa gene was cloned into expression vector pRSETb which directs expression of the foreign protein by the T7 promoter. The mature gene fragment was PCR amplified using primers J and K (Table 3) with pPX7760b as template DNA. The band  
 25 was cloned into PCR2.1 (pPX7792). pPX7792 was cleaved with NdeI and EcoRV, and the 2 kb gene fragment was ligated into pRSETb cut with the same enzymes. After verification of the correct clone by restriction endonuclease digestion, the resulting plasmid, designated pPX7796, was transformed into the expression strain BLR(DE3)pLysS, selecting for ampicillin and chloramphenicol resistance. The strain  
 30 was grown to mid-logarithmic phase in HYSOY broth (Difco, Detroit, Mich.) containing ampicillin and chloramphenicol, and expression was induced by the addition of 1 mM IPTG. Expression proceeded for 2 hours, after which the cells were harvested by centrifugation. Whole cells lysates were run on 10-12% SDS-PAGE. The 77 kDa

protein band was overexpressed as determined by coomassie stained gels, and reacted with polyclonal serum raised against the native protein.

### 10.2 Purification of the recombinant 77 kDa protein.

- 5 Bacterial cells (ca. 32 g wet wt of *E. coli* BLR(DE3)pLysS (pPX7796) were resuspended in 150 ml of 10 mM NaPO<sub>4</sub> / 150 mM NaCl / 5.0 mM EDTA / 1.0 mM Pefabloc (pH 7.2) by homogenization using a Ultra-Turrex tissue homogenizer. The cells were disrupted using a Microfluidizer Model 110Y. Inclusion bodies containing the recombinant 77 kDa protein were isolated by centrifugation at 10,000 rpm using a Sorvall SLA-1500 rotor for 30 min at 10°C. Following centrifugation, the pellet was resuspended by homogenization in 150 ml of 10mM NaPO<sub>4</sub> / 150mM NaCl / 5.0 mM EDTA / 1.0% TX-100 (pH 7.2) and stirred for 2 hr at 4°C. The suspension was centrifuged at 10,000 rpm using a Sorvall SS-34 rotor for 30 min at 10°C. Following centrifugation, the pellet was resuspended by homogenization in 75 ml of 10mM NaPO<sub>4</sub> / 150mM NaCl / 5.0 mM EDTA / 1.0% Z 3-16 (pH 7.2) and stirred 15 overnight at room temp. The suspension was centrifuged at 10,000 rpm using a Sorvall SS-34 rotor for 30 min at 10°C. Following centrifugation, the supernatant containing the recombinant 77 kDa protein was collected and stored at -20°C for further purification. The Z 3-16 extract was buffer exchanged by passage over a 180 ml Sephadex G-25 (coarse) column equilibrated in 0.02 M Tris-HCl / 5.0 mM EDTA / 1.0% Z 3-14 (pH 8.0). The 20 buffer exchanged Z 3-16 crude extract preparation was loaded onto a 10 ml SP-Sephrose Fast Flow column equilibrated 0.02 M Tris-HCl / 5.0 mM EDTA / 1.0% Z 3-14 (pH 8.0). Unbound protein was washed through the column with an additional 2 column volumes of equilibration buffer. The recombinant 77 kDa protein was eluted using a linear NaCl gradient (0-0.5 M NaCl) in 0.02 M Tris-HCl / 5.0 mM EDTA / 1.0% Z 3-14 (pH 7.0) over 8 25 column volumes. Fractions were screened for recombinant 77 kDa protein by SDS-PAGE and Western analysis and pooled. Pooled fractions were dialyzed into PBS / 1.0% Z 3-14 overnight at 4°C.

### Example 11

- 30 11.1 Cloning and expression of the 79 kDa gene from *H. pylori* strain PBCC 1107  
The 79 kDa gene was cloned from 3 strains of *H. pylori*, PBCC 1107, Let 13, and SS1. Primers J and K (Table 3) were used to amplify the mature coding region for cloning into pRSETb following the same protocol as for the 75 and 77 kDa constructs.

WO 00/00614

PCT/US99/14375

Following transformation into BLR(DE3) pLysS and induction by IPTG, the recombinant plasmid pPX5048 directed the overexpression of the 79 kDa protein. The recombinant protein was easily detected in coomassie stained gels and reacted with both polyclonal sera raised against native 75, and 77 kDa genes (due to its homology) and  
 5 with peptide-conjugate antisera directed against a sequence unique to the 79 kDa protein (described below).

### 11.2 Purification of the recombinant 79 kDa protein.

Bacterial cells (ca. 15 g wet wt of *E. coli* BL21(DE3)pLysS / pPX5048) are resuspended in  
 10 75 ml of 10 mM NaPO<sub>4</sub> / 150 mM NaCl / 5.0 mM EDTA / 1.0 mM Pefabloc (pH 7.2) by homogenization using a Ultra-Turrex tissue homogenizer. The cells are disrupted by sonication using a Branson Sonifier Cell Disrupter. Inclusion bodies containing the recombinant 79 kDa protein are isolated by centrifugation at 10,000 rpm using a Sorvall SS-31 rotor for 30 min at 10°C. Following centrifugation, the pellet is resuspended by  
 15 homogenization in 75 ml of 10mM NaPO<sub>4</sub> / 150mM NaCl / 5.0 mM EDTA / 4.0% TX-100 (pH 7.2) and stirred for 1.5 hr at 4°C. The suspension is centrifuged at 10,000 rpm using a Sorvall SS-31 rotor for 30 min at 10°C. Following centrifugation, the pellet is resuspended by homogenization in 75 ml of 10mM NaPO<sub>4</sub> / 150mM NaCl / 5.0 mM EDTA / 1.0% Z 3-14 (pH 7.2) and stirred for 1.5 hr at room temp. The suspension is centrifuged at 10,000  
 20 rpm using a Sorvall SS-34 rotor for 30 min at 10°C. Following centrifugation, the pellet is resuspended by homogenization in 75 ml of 10 mM NaPO<sub>4</sub> / 150 mM NaCl / 5.0 mM EDTA / 1.0% Z 3-16 (pH 7.2) and stirred for 2 hr at room temp. The suspension is centrifuged at 10,000 rpm using a Sorvall SS-31 rotor for 30 min at 10°C. Following centrifugation, the Z 3-14 and Z 3-16 supernatants containing the recombinant 79 kDa  
 25 protein are collected and stored at -20°C for further purification. The Z 3-14 extract is buffer exchanged by passage over a 180 ml Sephadex G-25 (coarse) column equilibrated in 0.02 M Tris-HCl / 5.0 mM EDTA / 1.0% Z 3-14 (pH 8.0). The buffer exchanged Z 3-14 crude extract preparation is loaded onto a 10 ml SP-Sepharose Fast Flow column equilibrated 0.02 M Tris-HCl / 5.0 mM EDTA / 1.0% Z 3-14 (pH 8.0). Unbound protein is  
 30 washed through the column with an additional 2 column volumes of equilibration buffer. The recombinant 79 kDa protein is eluted using a linear NaCl gradient (0-0.5 M NaCl) in 0.02 M Tris-HCl / 5.0 mM EDTA / 1.0% Z 3-14 (pH 7.0) over 8 column volumes. Fractions are screened for recombinant 79 kDa protein by SDS-PAGE and Western analysis and



pooled. Pooled fractions are dialyzed into PBS / 1.0% Z 3-14 overnight at 4°C. The Z 3-16 extract is processed as described for the Z 3-14 extract.

### Example 12

#### 5 DNA Sequence and Predicted Translation of 75, 77 and 79 kDa genes

The DNA sequence of the 75 kDa gene from strain ATCC 43579 corresponding to the mature protein is shown in Figure 6, SEQ ID NO. 21. The open reading frame is 2070 bp long, the same as the TIGR predicted protein. The translated protein is 689 aas long containing 6 cysteine residues with an estimated pI of 8.88 (Figure 7, SEQ ID NO. 19).

- 10 Alignment of the 75 kDa proteins from TIGR strain 26695 and ATCC 43579 reveals that 26 amino acid residues are not conserved.

The DNA sequence of the 77 kDa gene from strain ATCC 43579 is shown in SEQ ID NO. 5. The open reading frame corresponding to the mature protein is 2166 bp long, the same as the TIGR predicted protein. The translated protein is 721 aas long containing 6 cysteine residues with an estimated pI of 8.89 (SEQ ID NO. 2). Alignment of the 77 kDa proteins from TIGR strain 26695 and ATCC 43579 reveals substitutions in 67 aas. An additional 3 aas are unique to the TIGR protein, and an additional 11 aas are unique to the ATCC 43579 sequence.

20

The DNA sequence of the 79 kDa gene from strain PBCC 1107 is shown in Figure 8, SEQ ID NO. 22. The open reading frame corresponding to the mature protein is 2157 bp long, 21 bp shorter than the TIGR predicted protein. The translated protein from strain PBCC 1107 is 718 aas long containing 8 cysteine residues with an estimated pI of 6.99 which is considerably less than the pI of 8.68 predicted by the TIGR sequence (Figure 9, SEQ ID NO. 20). Alignment of the 79 kDa proteins from the TIGR strain 26695 and PBCC 1107 revealed 99 amino acid residue changes. There are 6 aas unique to the TIGR 79 kDa sequence, and 1 aa unique to the PBCC 1107 79 kDa sequence.

25

#### 30 Example 13

**Comparison of expression constructs for the overproduction of the 77, 75 and 79kd proteins of *H. pylori***

WO 00/00614

PCT/US99/14375

13.1 - 77K. The full length gene was cloned into pET17b using the NdeI site to fuse the 5' ends of the 77K gene to the vector ATG start codon, resulting in the full unaltered gene fused to the strong T7 promoter (pPX7762, Table 4). The full length gene was also cloned into an arabinose expression vector which also has strong promoter and which is tightly regulated. In addition, a third construct cloned the mature 77K gene to an ompT leader in pET12b which could enhance the translation and signal processing of the mature 77K gene (pPX7782). None of the constructs containing the gene with a signal sequence were overexpressed in excess of 1% total cellular protein (Table 4).

10 Primers was made corresponding to the start of the mature protein and the end of the gene (Table 3, primers D and E). DNA sequence corresponding to the mature protein was PCR amplified using the pPX7760b as template DNA and cloned into PCR2.1 (pPX7769). The gene was cleaved from pPX7769 by digestion with Asp718 and SpeI and inserted into the T7 promoted pET17b (Novagen, Madison, Wisc.) at the Asp718 and SpeI sites. The resulting plasmid fused 18 amino acid residues from the vector to the mature 77 kDa gene. Moderate expression of the 77 kDa protein was observed, but the induced protein was not visible by coomassie stained gels, indicating that the recombinant protein constituted less than 1 % of the total cellular protein. The mature gene fragment was also cloned into the arabinose expression vector pBAD24, which provided the ATG methionine and two additional vector-encoded amino acids to the mature gene. The recombinant plasmids were housed in the appropriate *E. coli* strains, BLR(DE3) pLysS for T7 promoted vectors and Top10F(ara-) for arabinose vectors. Overnight cultures were seeded into HYSOY media containing 0.5-1.0% glycerol and the appropriate antibiotic for selection. Cultures in mid-logarithmic phase were induced either with 1 mM IPTG (T7 vectors) or 0.0002-0.2% L-arabinose (arabinose vectors) and induction was allowed to proceed for 2 hours. Cells were harvested and resuspended in 1X SDS-PAGE loading buffer and run on a 12% SDS-PAGE gel. The pBAD24 construct produced the recombinant 77 kDa protein which was detected by Western blot but not by coomassie stained gels.

30

Figure 11 shows that the 77K protein expressed from pE717b was not above 1% of the total cellular protein. The pRSETb based clone, pPX7796, was able to overexpress the protein greater than 10-20% of total cellular protein. Analysis of the

differences between pET17b and pRSETb reveals that both vectors have identical promoter sequences. A significant difference between the two vectors is in the copy number. pRSET b is based on the pUC replicon which maintains 100-700 copies per cell whereas pET vectors are based on a pMB1/ColE1 replicon maintaining 25-30  
5 copies per cell. A strong promoter coupled with a relatively high gene dosage resulted in overproduction of the recombinant mature 77K protein.

Example -75K. The mature gene (pPX7768) was amplified with primers D and E (Table 3) to obtain a fragment with appropriate restriction site ends for cloning into the  
10 arabinose expression vector pBAD24 (Guzman et al. 1995. J. Bacteriol. 177:4121-4130), fusing the vector ATG start codon and two additional vector encoded amino acids to the mature gene (pPX7794). The 75 kDa gene was expressed by this vector following induction with arabinose, but the level was approximately 1% of cellular protein and the recombinant protein was prone to proteolytic cleavage. The mature  
15 coding region was cloned into the T7 polymerase expression system pRSETb (Invitrogen, San Diego Ca) by amplifying the 75 kDa gene from pPX7768 using primers J and K (Table 3) and ligating the fragment into the NdeI and EcoRV sites of pRSETb. The resulting recombinant plasmid (pPX7811) fuses the vector ATG to the mature 75 kDa coding sequences. When the recombinant plasmid was induced by IPTG in the  
20 BLR(DE3) pLysS expression strain (Novagen, Madison, Wisc.), the recombinant protein was approximately 10% of the cell mass and was resistant to proteolysis. Comparison of recombinant protein yields after induction reveals that pPX7811 was clearly superior in overexpressing the 75K mature protein (Figure 11).

Example -79K The mature 79K gene from strain PBCC1107 was cloned into the arabinose vector pBAD24 fusing the vector ATG and 3 other amino acids to the start of the 75K gene (pPX5043, Table 4) and to the pRSET vector, fusing the vector ATG to the start of the mature 75K gene (pPX5048, Table). Comparison of recombinant protein yields after induction reveals that pPX5048 was superior in overexpressing the 79K  
30 mature protein (Figure 12).

**Notes on Recombinant protein induction and purification for the production of 75, 77, 79K proteins**

For the production of high yield of recombinant protein, the ampicillin marker used in plasmids pPX7796, pPX7811 and pPX5043 is partially deleted and a gene for Kanamycin resistance is cloned in the site. This allows for fermentation of the proteins for use in humans. Strain *E. coli* BLR(DE3)pLysS housing either pPX7796, pPX7811 or pPX5043 is grown in HYSOY media containing glycerol until logarithmic phase and induced with 0.5-1 mM IPTG. Induction proceeds for 2-4 hours after which the bacteria is harvested by centrifugation.

Table 3A

| Primer | Description                                        | SEQ. ID NO. |
|--------|----------------------------------------------------|-------------|
| A      | 5' start of 77 and 79 kDa genes (sense)            | 8           |
| B      | 3' end of 75, 77, and 79 kDa genes (antisense)     | 9           |
| C      | Promoter region of 75 kDa gene (sense)             | 10          |
| D      | 5' start mature 75, 77, and 7kDa genes (sense)     | 11          |
| E      | 3' end of 75, 77, and 7kDa genes (antisense)       | 12          |
| F      | Internal 75 kDa specific (sense)                   | 13          |
| G      | Internal 75 kDa specific (antisense)               | 14          |
| H      | Internal 75 kDa seamless cloning (sense)           | 15          |
| I      | Internal 7kDa seamless cloning (antisense)         | 16          |
| J      | 5' end start mature 75, 77, and 7kDa genes (sense) | 17          |
| K      | 3' end of 75, 77, and 7kDa genes (antisense)       | 18          |

Table 3B

| Primer | Sequence (5' to 3')                          | SEQ. ID NO. |
|--------|----------------------------------------------|-------------|
| A      | GGCCATATGAAAAAACACATCCTTTCATTAGCT            | 8           |
| B      | GGCAAGCTTGGGAGTTTCACAAAAAGCTTAGTAAGCGAACAC   | 9           |
| C      | CGAAATCTTGTGATAAGATC                         | 10          |
| D      | CCGGGCTTGGTACCGGAAGACGACGGCTTTTAC            | 11          |
| E      | CCGGGCACTAGTTTAGTAAGCGAACACATAATTCAAATACACGC | 12          |
| F      | CGGGGGGCAAACCAAATACAC                        | 13          |
| G      | GTGTATTGGTTTGCCCCCG                          | 14          |
| H      | GCCGAACGTCTCCCCTAAATGAGGCATGCCCAAAC          | 15          |
| I      | CTACGGCGTCTCTTAGGGTAGTGATAATGATGCTCGCTTG     | 16          |
| J      | CGCGGCGATATGGAAGACGACGGCTTTTAC               | 17          |
| K      | CCGGCCGGTACCTTAGTAAGCGAACACATA               | 18          |

**WO 00/00614**

**PCT/US99/14375**

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Table 4 - Description of recombinant 75K, 77 K, 79 K clones.

| Plasmid | Gene       | Vector (promoter)  | Cell line       | Description                                              |
|---------|------------|--------------------|-----------------|----------------------------------------------------------|
| pPX7762 | full 77K   | pET17b (T7)        | BL21(DE3) pLysS | full length 77K gene fused to vector ATG                 |
| pPX7772 | signal-77K | pET17b (T7)        | BL21(DE3) pLysS | mature 77K gene N terminal fusion to T7Tag               |
| pPX7779 | full 77K   | pBAD24 (arabinose) | TOP10           | full length 77K gene fused to vector ATG                 |
| pPX7782 | signal-77K | pBAD24 (arabinose) | TOP10           | mature 77K gene fused to vector ATG, 3 aas on N terminus |
| PPX7781 | signal-77K | pET12b (T7)        | BL21(DE3) pLysS | mature 77K gene fused to vector OmpT leader              |
| PPX7796 | signal-77K | pRSETb (T7)        | BLR(DE3) pLysS  | mature 77K gene fused to vector ATG                      |
| PPX7794 | signal-75K | pBAD24 (arabinose) | TOP10           | mature 77K gene fused to vector ATG, 3 aas on N terminus |
| PPX7811 | signal-75K | pRSETb (T7)        | BLR(DE3) pLysS  | mature 77K gene fused to vector ATG                      |
| PPX5043 | signal-79K | pBAD24 (arabinose) | TOP10           | mature 77K gene fused to vector ATG, 3 aas on N terminus |
| PPX5048 | signal-79K | pRSETb (T7)        | BLR(DE3) pLysS  | mature 77K gene fused to vector ATG                      |

**Example 14****Generation of peptide specific polyclonal antibodies to  
the 77 and 79 kDa proteins from *H. pylori***

5

**14.1 Summary.**

Ten peptides representing unique sequences of the 77 and 79 kDa proteins (five to each) from *H. pylori* were conjugated to CRM197. CRM197 is a well known carrier protein as described in Uchida, T. et al., 1971 Nature New Biology, Vol. 233, 8-11. All of the  
10 conjugates were characterized by SDS-PAGE and amino acid analysis. The soluble conjugates were characterized by MALDI-TOF mass spectrometry analysis. An animal study was performed using the peptide conjugates as the antigen in order to produce antisera that would specifically recognize either the 77 or 79 kDa protein of *H. pylori* by western blot for purification protocols.

15

**14.2 Peptides Used For Conjugation.**

All peptides were synthesized using the Gilson Multiple Peptide Synthesizer with a N-terminal cysteine residue necessary for covalent attachment to the carrier CRM197 protein in the specific conjugation chemistry used. The amino acid sequences of the  
20 peptides representing the 77 and 79 kDa peptides can be found in Table 5. All peptides were purified to near homogeneity by reversed phased HPLC and characterized by MALDI-TOF mass spectrometry and amino acid composition analysis.

**14.3 Preparation of Peptide-CRM197 Conjugates.**

25

The 77 and 79 kDa peptides were conjugated to CRM197 using the crosslinking reagent N-succinimidyl bromoacetate (Bernatowicz et al.). On the day of the conjugation, the peptides were reacted with 5,5'-dithio bis(2-nitrobenzoic acid) [Ellman's reagent] to verify the content of free SH groups (resulted in greater than 95% free SH groups). Free amino groups of CRM197 were bromoacetylated by reaction with an excess of  
30 bromoacetic acid N-hydroxysuccinimide ester (Sigma Chemical Co.). To an ice cold solution of CRM197, 10% (v/v) 1.0 M NaHCO<sub>3</sub> (pH 8.4) was added. Bromoacetic acid N-hydroxysuccinimide ester, equal in weight to that of CRM197 used, was dissolved in 200  $\mu$ l dimethylformamide, added slowly to the CRM197 and gently mixed at room temperature in the dark for 1 hour. The resulting bromoacetylated (activated) protein

was purified by passage through a P6-DG column using PBS, 1 mM EDTA (pH 7.0) as the eluent. Following purification, the fractions corresponding to activated CRM<sub>197</sub> were pooled and the protein concentration was estimated by BCA protein assay (Pierce Chemical Co.). To determine the extent of bromoacetylation, the protein amino groups, both before and after treatment with bromoacetic acid N-hydroxysuccinimide ester, were reacted with 2,4,6-trinitrobenzenesulfonic acid (TNBS). Approximately 70-75% of the amino groups were found to be bromoacetylated (activated). Following TNBS assay, approximately 2.4 – 4.6 mg of peptide was dissolved in sterile distilled water to an approximate concentration of 20 mg/ml. The peptide was slowly added to cold activated CRM<sub>197</sub> in a 1:1 ratio (w/w) and the pH was adjusted to approximately 7.0-7.2. The resulting material was gently mixed overnight at 4°C in the dark followed by dialysis in the dark against two 1 L changes of PBS, pH 7.2. Each conjugate was then transferred into a sterile 15 ml polypropylene tube, wrapped in aluminum foil and stored at 4°C.

15

#### 14.4 Characterization of Peptide-CRM<sub>197</sub> Conjugates.

To verify conjugation, all peptide-CRM<sub>197</sub> conjugates were analyzed by amino acid analysis (for the presence of the characteristic S-carboxymethylcysteine residues) and SDS-PAGE. The soluble conjugates and activated CRM<sub>197</sub> were subjected to MALDI-TOF mass spectrometry analysis. The protein concentration was determined by the average of values determined by amino acid analysis and BCA (Table 6).

20

##### 14.4(a) Amino Acid Analysis of Peptide-CRM<sub>197</sub> Conjugates.

A suitable aliquot of each conjugate was hydrolyzed using 6N HCl in the presence of 5% phenol (v/v) and 1% 2-mercaptoethanol (v/v) under vacuum for 22 hours at 110°C. Following evaporation and resolubilization in Beckman sodium citrate sample dilution buffer (pH 2.2), the samples were analyzed on a Beckman 6300 Amino Acid Analyzer according to manufacturer's instructions. The degree of conjugation was determined by the estimated amount of S-carboxymethylcysteine residues formed per mole of CRM<sub>197</sub>. The degree of conjugation for each conjugate is described in Table 7.

30

##### 14.4(b) SDS-PAGE Analysis of Peptide-CRM<sub>197</sub> Conjugates



A suitable aliquot of each conjugates was mixed with reducing sample buffer and heated at 100°C for 5 minutes. The conjugates and Mark12 molecular weight (MW) standards (Novex) were loaded on a 10 % (w/v, acrylamide) gel and SDS-PAGE (Laemmli) was carried out. Following SDS-PAGE, the gel was stained with Coomassie brilliant blue and destained. The samples exhibited a smear staining pattern across a wide MW range that is characteristic of similar peptide conjugates.

#### 14.4(c) MALDI-TOF Mass Spectrometry Analysis of Peptide-CRM<sub>197</sub> Conjugates.

A suitable aliquot of activated CRM<sub>197</sub> and each soluble conjugate were analyzed by MALDI-TOF mass spectrometry using 3,5-dimethoxy-4-hydroxy-cinnamic acid (sinapinic acid) as the matrix. Due to the addition of bromoacetyl groups (120 mass units each) to 29 lys residues of CRM<sub>197</sub> (mol. wt. 58,408.7), which was based on approximately 75% activation from the TNBS assay, activated CRM<sub>197</sub> was expected to have a mol. wt. of  $[58,408.7 + (29 \times 120)] = 61,889$ . The experimental mol. wt. of activated CRM<sub>197</sub> determined by MALDI-TOF mass spectrometry was 61,208 for activated CRM<sub>197</sub> used for the 77k peptide conjugates and 61,009 for activated CRM<sub>197</sub> used for the 79k peptide conjugates. The degree of conjugation for each conjugate was calculated by subtracting the mass value of activated CRM<sub>197</sub> from the mass value of each conjugate and dividing by the mass of the peptide used to prepare the conjugate. The degree of conjugation for the soluble 77k and 79k peptide conjugates is described in the table 8. The mass values for heavily precipitated conjugates could not be determined.

#### 14.5 Animal Study.

An animal study using the peptide conjugates was performed to produce antisera that will differentially recognize the 77kDa protein from the 79kDa protein in *H. pylori* blot. Table 9 describes the protocol for the animal study.

#### 14.6 Analysis of Immune Sera by Peptide ELISA.

All peptide ELISAs were run using Nunc Maxisorp 96-well plates. Weeks 0 and 6 peptide conjugate antisera were all titrated against homologous peptides. Plates were coated with 100 µl of peptides diluted to 1.0 µg/ml with 50 mM sodium bicarbonate (pH

9.0) and incubated at 37°C overnight. Plates were washed and blocked for 1 hour at 37°C with 250 µl of 3% BSA in 137 mM tris buffered saline (TBS), pH 7.6. Peptide conjugate antisera were serially diluted into 0.3% BSA in TBS containing 0.05% Tween-20, 100 µl of each dilution was added to the plates and incubated at 37°C for 1 hour. The plates were washed and subsequently incubated for 1 hour at 37°C with 100 µl of alkaline phosphatase conjugated goat anti mouse secondary antibody (Zymed) diluted 1:1500 in 0.3% BSA in TBS containing 0.05% Tween-20. The plates were washed and finally incubated for 1 hour at room temperature with 100 µl p-nitrophenyl phosphate substrate prepared in diethanolamine containing 0.5 mM MgCl<sub>2</sub>. Plates were read using an automated 96-well plate reader with a 405 nm test and 690 nm reference filter. All endpoint titers were calculated at 60 minutes at 0.1 AU. Peptide ELISA endpoint titers are shown in table 6.

#### 14.7 Analysis of Immune Sera by Western blot

Week 6 sera from mice immunized with peptide groups: 97-7; #1, #6, #7 and 97-14; #14, #17 was used, (as stated in the Western Blot protocol section ) as the primary antibody in a Western blot against whole cell lysates of *Helicobacter pylori* and the recombinant 75, 77, and 79 kDa proteins. Conjugate 97-14 #14 generated a strong specific signal to the 79 kDa protein in both the *H.pylori* sample and the recombinant 79 kDa protein, but not to native or recombinant 75 and 77 kDa proteins.

**Table 5.** Sequences of peptides used in the 77 and 79 kDa conjugates.

WO 00/00614

PCT/US99/14375

| Conjugate                         | Peptide Sequence                |
|-----------------------------------|---------------------------------|
| 97-6 #3/CRM <sub>197</sub> (77k)  | CASGNTSHVITNKLDGVPDS (SEQ 23)   |
| 97-6 #7/CRM <sub>197</sub> (77k)  | CSPSVNGTKTTTQTIDGK (SEQ 24)     |
| 97-6 #9/CRM <sub>197</sub> (77k)  | CYFHATNSSEANAPKFS (SEQ 25)      |
| 97-6 #15/CRM <sub>197</sub> (77k) | CNPENLSENFKN (SEQ 26)           |
| 97-6 #20/CRM <sub>197</sub> (77k) | CSGQGNNN (SEQ 27)               |
| 97-7 #1/CRM <sub>197</sub> (79k)  | CVMKNNNNVNEKLAGFGKEEVM (SEQ 28) |
| 97-7 #6/CRM <sub>197</sub> (79k)  | CKAKNGSSSSSNGGNGSS (SEQ 29)     |
| 97-7 #11/CRM <sub>197</sub> (79k) | CTTTYNNNKATVKFDIT (SEQ 30)      |
| 97-7 #14/CRM <sub>197</sub> (79k) | CLVRSTNNENTPGGGQ (SEQ 31)       |
| 97-7 #17/CRM <sub>197</sub> (79k) | CRQTADINGGVYQF (SEQ 32)         |

**Table 6.** Concentration of 77 and 79kDa peptide conjugates. Concentration was determined by averaging the values determined by amino acid analysis and BCA.

| Conjugate                            | Concentration (mg/ml) |
|--------------------------------------|-----------------------|
| 97-6 #3/CRM <sub>197</sub> (77kDa)   | 3.5                   |
| 97-6 #7/CRM <sub>197</sub> (77kDa)   | 2.6                   |
| 97-6 #9/CRM <sub>197</sub> (77kDa)   | 3.8                   |
| 97-6 #15/CRM <sub>197</sub> (77kDa)  | 2.5                   |
| 97-6 #20/CRM <sub>197</sub> (77kDa)  | 3.2                   |
| 97-7 #1/CRM <sub>197</sub> (79kDa)   | 1.8                   |
| 97-7 #6/CRM <sub>197</sub> (79kDa)   | 0.9                   |
| 97-7 #11/CRM <sub>197</sub> (79kDa)  | 3.3                   |
| 97-14 #14/CRM <sub>197</sub> (79kDa) | 3.3                   |
| 97-14 #17/CRM <sub>197</sub> (79kDa) | 3.7                   |

**Table 7.** Degree of conjugation for each peptide conjugate based on amino acid analysis.

| Conjugate                            | Degree of Conjugation<br>(moles peptide per mole CRM <sub>197</sub> ) |
|--------------------------------------|-----------------------------------------------------------------------|
| 97-6 #3/CRM <sub>197</sub> (77kDa)   | 11                                                                    |
| 97-6 #7/CRM <sub>197</sub> (77kDa)   | 9                                                                     |
| 97-6 #9/CRM <sub>197</sub> (77kDa)   | 17                                                                    |
| 97-6 #15/CRM <sub>197</sub> (77kDa)  | 8                                                                     |
| 97-6 #20/CRM <sub>197</sub> (77kDa)  | 18                                                                    |
| 97-7 #1/CRM <sub>197</sub> (79kDa)   | 5                                                                     |
| 97-7 #6/CRM <sub>197</sub> (79kDa)   | 12                                                                    |
| 97-7 #11/CRM <sub>197</sub> (79kDa)  | 14                                                                    |
| 97-14 #14/CRM <sub>197</sub> (79kDa) | 13                                                                    |
| 97-14 #17/CRM <sub>197</sub> (79kDa) | 17                                                                    |

**Table 8.** Degree of conjugation of 77k and 79kDa peptide conjugates.

The mass of the conjugate is approximate due to the irregular shape of the peak (broad and sometimes rough) possibly representing a distribution of conjugates containing different amounts of peptides attached. ND = not determined due to precipitate.

5

| Conjugate                               | Mass of Conjugate<br>(Approximate) | Mass of Peptide | Degree of<br>Conjugation<br>(# peptides per<br>CRM <sub>197</sub> ) |
|-----------------------------------------|------------------------------------|-----------------|---------------------------------------------------------------------|
| 97-6 #3/CRM <sub>197</sub> (77kDa)      | 79,555                             | 2,013           | 9.1                                                                 |
| 97-6 #7/CRM <sub>197</sub> (77kDa)      | 74,121                             | 1,836           | 7.0                                                                 |
| 97-6 #9/CRM <sub>197</sub> (77kDa)      | 86,910                             | 1,872           | 13.7                                                                |
| 97-6 #15/CRM <sub>197</sub> (77kDa)     | 68,870                             | 1,407           | 5.4                                                                 |
| 97-6 #20/CRM <sub>197</sub> (77kDa)     | 72,036                             | 792             | 13.7                                                                |
| 97-7 #1/CRM <sub>197</sub> (79kDa)      | ND                                 | 2,466           | ND                                                                  |
| 97-7 #6/CRM <sub>197</sub> (79kDa)      | ND                                 | 1,627           | ND                                                                  |
| 97-7 #11/CRM <sub>197</sub> (79kDa)     | ND                                 | 1,932           | ND                                                                  |
| 97-14 #14/CRM <sub>197</sub><br>(79kDa) | 79,231                             | 1,645           | 11.1                                                                |
| 97-14 #17/CRM <sub>197</sub><br>(79kDa) | ND                                 | 1,570           | ND                                                                  |

WO 00/00614

PCT/US99/14375

**Table 9.** 77k and 79kDa peptide conjugate animal study protocol. Swiss Webster mice were used for entire study. Injection volume = 100 µl; B = bleed; V = vaccinate.

| Conjugate                               | Dose µg | Adjuvant    | Wk 0 | Wk 3 | Wk 6 | Wk 8 |
|-----------------------------------------|---------|-------------|------|------|------|------|
| 97-6 #3/CRM <sub>197</sub> (77kDa)      | 10      | 20 µg QS-21 | B, V | V    | B, V | B    |
| 97-6 #7/CRM <sub>197</sub> (77kDa)      | 10      | 20 µg QS-21 | B, V | V    | B, V | B    |
| 97-6 #9/CRM <sub>197</sub> (77kDa)      | 10      | 20 µg QS-21 | B, V | V    | B, V | B    |
| 97-6 #15/CRM <sub>197</sub><br>(77kDa)  | 10      | 20 µg QS-21 | B, V | V    | B, V | B    |
| 97-6 #20/CRM <sub>197</sub><br>(77kDa)  | 10      | 20 µg QS-21 | B, V | V    | B, V | B    |
| 97-7 #1/CRM <sub>197</sub> (79kDa)      | 10      | 20 µg QS-21 | B, V | V    | B, V | B    |
| 97-7 #6/CRM <sub>197</sub> (79kDa)      | 10      | 20 µg QS-21 | B, V | V    | B, V | B    |
| 97-7 #11/CRM <sub>197</sub><br>(79kDa)  | 10      | 20 µg QS-21 | B, V | V    | B, V | B    |
| 97-14 #14/CRM <sub>197</sub><br>(79kDa) | 10      | 20 µg QS-21 | B, V | V    | B, V | B    |
| 97-14 #17/CRM <sub>197</sub><br>(79kDa) | 10      | 20 µg QS-21 | B, V | V    | B, V | B    |

**Table 10.** Weeks 0 and 6 peptide ELISA endpoint titers of 77 and 79kDa peptide conjugate antisera against homologous peptides

| Conjugate                            | Week 0 Titer | Week 6 Titer |
|--------------------------------------|--------------|--------------|
| 97-6 #3/CRM <sub>197</sub> (77Da)    | 478          | 1,397,000    |
| 97-6 #7/CRM <sub>197</sub> (77kDa)   | 375          | 513,400      |
| 97-6 #9/CRM <sub>197</sub> (77kDa)   | 324          | 967,900      |
| 97-6 #15/CRM <sub>197</sub> (77kDa)  | 504          | 1,066,000    |
| 97-6 #20/CRM <sub>197</sub> (77kDa)  | 511          | 105,600      |
| 97-7 #1/CRM <sub>197</sub> (79kDa)   | 369          | > 2,187,000  |
| 97-7 #6/CRM <sub>197</sub> (79kDa)   | 468          | 120,400      |
| 97-7 #11/CRM <sub>197</sub> (79kDa)  | 492          | 1,438,000    |
| 97-14 #14/CRM <sub>197</sub> (79kDa) | 261          | 523,000      |
| 97-14 #17/CRM <sub>197</sub> (79kDa) | 2,242        | 1,644,000    |

5

**Example 15**

**Prophylactic and Therapeutic Mouse Models  
with 75/77 kDa Proteins**

10

**15.1 Prophylactic Mouse Model.**

C57BL/6 mice were vaccinated intragastrically with 100 µg native 75.77 kDa proteins admixed with 10 µg CT, 10 µg CT-E29H (an attenuated cholera toxin mutant as disclosed in U.S. Provisional Application No. 60/102,430 which is hereby incorporated herein), 100 µg CpG20mer (a CpG oligonucleotide sequence, see Davis et al., 1998. J. Immunol. 160:870-876), or 10 µg CT-E29H + 100 µg CpG20mer on days 0, 2, 14, and 16. Mice were challenged intragastrically with  $2 \times 10^8$  cfu *H. pylori* strain SS1 on day 31. Organisms recovered on days 58 and 59 are expressed as log<sub>10</sub> cfu per gram of stomach tissue +/- 1 standard error of the mean. The number of colony forming units recovered was reduced by approximately 1-2 logs in mice vaccinated with 75/77 kDa protein admixed with CT, CT-E29H, and with CT-E29H + CpG20mer (Table 13).

20



**15.1(a) Immunogenicity of native 75/77 kDa proteins.**

Pre-challenge pooled sera (day 30) from mice vaccinated as above were analyzed for the presence of antibodies to the native 75/77 kDa proteins by standard ELISA (Table 11).

- 5 Samples containing mucosal antibodies were also screened for specific antibodies to the 75/77 kDa proteins (Table 12).

**15.2 Therapeutic Mouse Model.**

- C57BL/6 mice were infected intragastrically with  $2 \times 10^8$  cfu *H. pylori* strain SS1 on day 0. For intragastric vaccinations, mice received native 100 µg 75/77 kDa proteins, recombinant urease, or KLH admixed with CT on days 31, 33, 45, and 47. For subcutaneous vaccinations, mice were injected with 10 mg native 75/77 proteins admixed with AlPO<sub>4</sub> on days 32 and 60. A challenged but unvaccinated control group was included to assess the role of nonspecific clearance from CT in the KLH/CT group.
- 15 Recovery of organisms 2 and 4 wks after the last vaccination is expressed in log 10 cfu per gram of stomach tissue +/- 1 standard deviation. The number of colony forming units recovered was significantly reduced at the 2 wk time point for mice receiving native 75/77 kDa proteins intragastrically admixed with CT, and at the 4 wk time point for mice receiving native 75/77 kDa proteins subcutaneously admixed with AlPO<sub>4</sub>
- 20 (Figure 14).

**Example 16****16.1 Vaccine Characterization of Recombinant Proteins 75, 77 and 79.**

- The above protocols for the animal studies are repeated for each of the proteins to establish the specific attributes by each protein as a vaccine.
- 25

Table 11: Serum antibody responses to Hp n75/77kDa proteins administered intragastrically with various adjuvants in C57BL/6 mice

| Group <sup>a</sup> | Antigen <sup>b</sup> | Adjuvant <sup>c</sup> | anti n75/77kDa ELISA endpoint titers on pooled sera <sup>d</sup> |         |        |        |        |      |
|--------------------|----------------------|-----------------------|------------------------------------------------------------------|---------|--------|--------|--------|------|
|                    |                      |                       | IgA                                                              | IgG     | IgG1   | IgG2a  | IgG2b  | IgG3 |
| AA957              | KLH                  | CT                    | <100                                                             | 327     | nd     | nd     | nd     | nd   |
| AA958              | 75/77kDa             | CT                    | 1,279                                                            | 136,379 | 26,682 | 18,133 | 33,422 | 268  |
| AA959              | 75/77kDa             | E29H                  | 1,829                                                            | 134,188 | 33,387 | 4,352  | 21,158 | 513  |
| AA960              | 75/77kDa             | CpG20mer              | 2,009                                                            | 91,713  | 1,218  | 27,290 | 36,414 | 400  |
| AA961              | 75/77kDa             | E29H+CpG              | 826                                                              | 149,199 | 3,250  | 31,979 | 50,574 | 205  |

a. C57BL/6 mice, n = 12 per group.

b. 100 delivered IG in NaHCO<sub>3</sub> buffer on days 0, 2, 14, and 16..

c. adjuvanted with 10 µg CT, 10 µg E29H, 100 µg CpG20mer or 10 µg E29H + 100 µg CpG20mer.

d. pooled sera (n=12) on day 30, nd = not done.

Table 12: Mucosal antibody responses to Hp n75/77kDa proteins administered intragastrically with various adjuvants in C57BL/6 mice

| anti n75/77kDa ELISA endpoint titers on pooled mucosal samples <sup>d</sup> |                      |                       |     |     |              |     |        |     |              |       |       |  |
|-----------------------------------------------------------------------------|----------------------|-----------------------|-----|-----|--------------|-----|--------|-----|--------------|-------|-------|--|
| Group <sup>a</sup>                                                          | Antigen <sup>b</sup> | Adjuvant <sup>c</sup> | BAL |     | Fecal Pellet |     | Saliva |     | Vaginal Wash |       |       |  |
|                                                                             |                      |                       | IgA | IgG | IgA          | IgG | IgA    | IgG | IgA          | IgG   |       |  |
| AA957                                                                       | KLH                  | CT                    | <10 | <10 | 262          | 40  | <10    | <10 | <10          | <10   | <10   |  |
| AA958                                                                       | 75/77kDa             | CT                    | 10  | 215 | 106          | 18  | 52     | 69  | 398          | 295   | 295   |  |
| AA959                                                                       | 75/77kDa             | E29H                  | 194 | 720 | 409          | 92  | 33     | 78  | 238          | 652   | 652   |  |
| AA960                                                                       | 75/77kDa             | CpG20mer              | 347 | 379 | 568          | 86  | 585    | 147 | 147          | 242   | 242   |  |
| AA961                                                                       | 75/77kDa             | E29H+CpG              | 37  | 598 | 181          | 40  | 54     | 231 | 76           | 3,879 | 3,879 |  |

a. C57BL/6 mice, n = 12 per group.

b. 100 µg delivered IG in NaHCO<sub>3</sub> buffer on days 0, 2, 14, and 16.

c. adjuvanted with 10 µg CT, 10 µg E29H, 100 µg CpG20mer or 10 µg E29H + 100 µg CpG20mer.

d. pooled fecal pellets (n=12) on day 30 and pooled bronchoalveolar washes, saliva, and vaginal washes (n=4) on day 31.

Note: gram (g) amounts noted above are micrograms.

## REFERENCES

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We claim:

1. An isolated, substantially purified polypeptide comprising a  
5 polypeptide of *H. pylori* selected from the group consisting of:

(i) a polypeptide having a molecular weight of about 75 kDa;

(ii) a polypeptide having a molecular weight of about 77 kDa; and  
10

(iii) a polypeptide having a molecular weight of about 79 kDa;

wherein each polypeptide has the amino-terminal amino acid sequence  
EDDGFYTSVGYQIGEEAAQMV (SEQ ID No.: 7).  
15

2. The purified polypeptide of claim 1 wherein the polypeptide of *H. pylori* is a polypeptide having a molecular weight of about 75 kDa.

3. The purified polypeptide of claim 1 wherein the polypeptide of *H. pylori* is a polypeptide having a molecular weight of about 77 kDa.  
20

4. The purified polypeptide of claim 1 wherein the polypeptide of *H. pylori* is a polypeptide having a molecular weight of about 79 kDa.

5. An isolated polypeptide of *H. pylori* selected from the group  
25 consisting of:

(i) a polypeptide having a molecular weight of about 75 kDa and having  
the amino acid sequence of SEQ. ID NO.1 or the amino acid sequence of SEQ. ID  
30 NO.19;

(ii) a polypeptide having a molecular weight of about 77 kDa and having the amino acid sequence of SEQ. ID NO.2; and

5 (iii) a polypeptide having a molecular weight of about 79 kDa and having the amino acid sequence of SEQ. ID NO.3 or the amino acid sequence of SEQ. ID NO. 20;

6. The isolated polypeptide of claim 5 wherein the polypeptide has a molecular weight of about 75 kDa.

10

7. The isolated polypeptide of claim 5 wherein the polypeptide has a molecular weight of about 77 kDa.

8. The isolated polypeptide of claim 5 wherein the polypeptide has a molecular weight of about 79 kDa.

15

9. An antigenic composition of a substantially purified polypeptide comprising an isolated, substantially purified polypeptide of *H. pylori* selected from the group consisting of:

20

(i) a polypeptide having a molecular weight of about 75 kDa;

(ii) a polypeptide having a molecular weight of about 77 kDa; and

25 (iii) a polypeptide having a molecular weight of about 79 kDa;

wherein each polypeptide has the amino-terminal amino acid sequence of EDDGFYTSVGYQIGEEAQMV (SEQ ID No.: 7) and

30 (b) one or more of a pharmaceutically acceptable buffer, or diluent, adjuvant or carrier.

10. The antigenic composition of claim 9 wherein the carrier is conjugated to said polypeptide.

5 11. The antigenic composition of claim 9 wherein said adjuvant is a mucosal adjuvant.

12. The antigenic composition of claim 9 wherein said adjuvant comprises a liquid.

10

13. A vaccine comprising an antigenic composition comprising an immunologically effective amount of an isolated, substantially purified polypeptide of *H. pylori* selected from the group consisting of:

15 (i) a polypeptide having a molecular weight of about 75 kDa;

(ii) a polypeptide having a molecular weight of about 77 kDa; and

(iii) a polypeptide having a molecular weight of about 79 kDa;

20

wherein each polypeptide has the amino-terminal amino acid sequence EDDGFYTSVGYQIGEEAQMV (SEQ ID No.: 7); and

(b) one or more of a pharmaceutically acceptable buffer, diluent, adjuvant or carrier.

25

14. A method of inducing an immune response in a mammal which comprises administering to said mammal an immunologically effective amount of the antigenic composition of claim 9.

30



15. An isolated and purified nucleic acid sequence comprising a nucleotide sequence which hybridizes under high stringency Southern hybridization conditions with a nucleic acid sequence encoding a polypeptide selected from the group consisting of:

5

- (i) a polypeptide having a molecular weight of about 75 kDa;
- (ii) a polypeptide having a molecular weight of about 77 kDa; and
- (iii) a polypeptide having a molecular weight of about 79 kDa;

10

wherein each polypeptide has the amino-terminal amino acid sequence EDDGFYTSVGYQIGEEAQMV (SEQ ID No.: 7).

15

16. The isolated and purified nucleic acid sequence of claim 15, wherein said nucleotide sequence hybridizes under high stringency southern hybridization conditions with a nucleic acid having the nucleotide sequence of nucleotides 58-2124 of SEQ ID No.: 4 or the nucleotide sequence of nucleotides 1-2070 of SEQ ID No.: 21.

20

17. The isolated and purified nucleic acid sequence of claim 16, wherein said nucleic acid sequence has the nucleotide sequence of nucleotides 58-2124 of SEQ ID No. 4 or the nucleotide sequence of nucleotides 1-2070 of SEQ ID No.: 21.

25

18. An isolated and purified nucleic acid sequence comprising a nucleotide sequence encoding the polypeptide having the amino acid sequence of amino acids 20-708 of SEQ ID No. 1, the amino acid sequence of amino acids 1-689 of SEQ ID No. 19, or biologically equivalent amino acid sequence thereof.

30

19. The isolated and purified nucleic acid sequence of claim 15, wherein said nucleotide sequence hybridizes under high stringency southern hybridization

conditions with a nucleic acid having the nucleotide sequence of nucleotides 61-2223 of SEQ ID No.: 5.

20. The isolated and purified nucleic acid sequence of claim 19, wherein  
5 said nucleotide sequence hybridizes under high stringency southern hybridization conditions with a nucleic acid having the nucleotide sequence of nucleotides 61-2223 of SEQ ID No.: 5.

21. An isolated and purified nucleic acid sequence comprising a  
10 nucleotide sequence encoding the polypeptide having the amino acid sequence of amino acids 21-741 of SEQ ID No. 2, or biologically equivalent amino acid sequence thereof.

22. The isolated and purified nucleic acid sequence of claim 15, wherein  
15 said nucleotide sequence hybridizes under high stringency southern hybridization conditions with a nucleic acid having the nucleotide sequence of nucleotides 61-2235 of SEQ ID No.: 6 or the nucleotide sequence of nucleotides 1-2157 of SEQ ID No.: 22.

23. The isolated and purified nucleic acid sequence of claim 22, wherein  
20 said nucleic acid sequence has the nucleotide sequence of nucleotides 61-2235 of SEQ ID No.: 6 the nucleotide sequence of nucleotides 1-2157 of SEQ ID No.: 22.

24. An isolated and purified nucleic acid sequence comprising a  
nucleotide sequence encoding the polypeptide having the amino acid sequence of  
25 amino acids 21-745 of SEQ ID No. 3, or biologically equivalent amino acid sequence thereof or the amino acid sequence of amino acids 1-718 of SEQ ID No. 20.

25. A plasmid containing an isolated and purified nucleic acid sequence  
comprising a nucleotide sequence which hybridizes under high stringency Southern  
30 hybridization conditions with a nucleic acid sequence encoding a polypeptide selected from the group consisting of:

- (i) a polypeptide having a molecular weight of about 75 kDa;
- (ii) a polypeptide having a molecular weight of about 77 kDa; and
- 5 (iii) a polypeptide having a molecular weight of about 79 kDa;

wherein each polypeptide has the amino-terminal amino acid sequence  
EDDGFYTSVGYQIGEEAAQMV (SEQ ID No.: 7).

10

26. The plasmid of claim 25 wherein the plasmid contains an isolated and purified nucleic acid sequence, wherein said nucleotide sequence hybridizes under high stringency southern hybridization conditions with a nucleic acid having the nucleotide sequence of nucleotides 58-2124 of SEQ ID No.: 4 or the nucleotide  
15 sequence of nucleotides 1-2070 of SEQ ID No.: 21.

27. The plasmid of claim 26 wherein the plasmid contains an isolated and purified nucleic acid sequence, wherein said nucleic acid sequence has the nucleotide sequence of nucleotides 58-2124 of SEQ ID No. 4 the nucleotide sequence of  
20 nucleotides 1-2070 of SEQ ID No.: 21.

28. The plasmid of claim 25 wherein the plasmid contains an isolated and purified nucleic acid sequence comprising a nucleotide sequence encoding the polypeptide having the amino acid sequence of amino acids 20-708 of SEQ ID No. 1,  
25 the amino acid sequence of amino acids 1-689 of SEQ ID No. 19, or biologically equivalent amino acid sequence thereof.

29. The plasmid of claim 25 wherein the plasmid contains an isolated and purified nucleic acid sequence, wherein said nucleotide sequence hybridizes under  
30 high stringency southern hybridization conditions with a nucleic acid having the nucleotide sequence of nucleotides 61-2223 of SEQ ID No.: 5.

30. The plasmid of claim 29 wherein the plasmid contains an isolated and purified nucleic acid sequence, wherein said nucleotide sequence has the nucleotide sequence of nucleotides 61-2223 of SEQ ID No.: 5.

5

31. The plasmid of claim 25 wherein the plasmid contains an isolated and purified nucleic acid sequence comprising a nucleotide sequence encoding the polypeptide having the amino acid sequence of amino acids 21-741 of SEQ ID No. 2, or biologically equivalent amino acid sequence thereof.

10

32. The plasmid of claim 25 wherein the plasmid contains an isolated and purified nucleic acid sequence, wherein said nucleotide sequence hybridizes under high stringency southern hybridization conditions with a nucleic acid having the nucleotide sequence of nucleotides 61-2235 of SEQ ID No.: 6 or the nucleotide sequence of nucleotides 1-2157 of SEQ ID No.: 22.

15

33. The plasmid of claim 32 wherein the plasmid contains an isolated and purified nucleic acid sequence of claim 22, wherein said nucleic acid sequence has the nucleotide sequence of nucleotides 61-2235 of SEQ ID No.: 6 or the nucleotide sequence of nucleotides 1-2157 of SEQ ID No.: 22.

20

34. The plasmid of claim 25 wherein the plasmid contains an isolated and purified nucleic acid sequence comprising a nucleotide sequence encoding the polypeptide having the amino acid sequence of amino acids 21-745 of SEQ ID No. 3, the amino acid sequence of amino acids 1-718 of SEQ ID No. 20 or biologically equivalent amino acid sequence thereof.

25

35. The method of claim 14 wherein said composition is administered parenterally.

30

36. The method of claim 14 wherein said composition is administered orally.
37. The method of claim 1 wherein the polypeptides are co-purified.
- 5 38. The plasmid of claim 25 wherein the plasmid is a high copy number plasmid generating at least about 100 copies per cell.
- 10 39. The plasmid of claim 25 wherein the plasmid is a high copy number plasmid generating from about 100 to 1,000 copies per cell.
40. The plasmid of claim 25 wherein the plasmid is a high copy number plasmid generating from about 100 to 700 copies per cell.
- 15 41. The plasmid of claim 40 wherein a nucleic acid sequence encoding a selected polypeptide is operably linked to a strong promoter.
42. The plasmid of claim 40 wherein the promoter is selected from the group consisting of T7 promoter, arabinose, lambda phage promoters, tac and trc promoters.
- 20 43. The plasmid of claim 25 wherein the nucleic acid sequence encoding a selected polypeptide is expressed in at least about 5 to about 50% of the cellular protein of a host cell
- 25 44. The plasmid of claim 25 wherein the nucleic acid sequence encoding a selected polypeptide is expressed in at least about 10 to about 40% of the cellular protein of a host cell

45. The plasmid of claim 25 wherein the nucleic acid sequence encoding a selected polypeptide is expressed in at least about 10 to about 30% of the cellular protein of a host cell

5 46. The plasmid of claim 43 wherein the nucleotide sequence encodes the a mature portion the desired polypeptide

47. A host cell transformed with at least one plasmid of claims 25-34 and claims 38-46.

10

48. A method of producing an *H. pylori* polypeptide which comprises transforming or transfecting a host cell with at least one the plasmid of claims 25-34 and claims 38-46, and then culturing the host cell under conditions which permit the expression of said polypeptide by the host cell.

15

49. The host cell of claim 47 wherein the cell is a bacterial cell.

50. The method of claim 48 wherein the host cell is a bacterial cell.

20 51. A polyclonal antibody which preferentially binds to one polypeptide of *H. pylori* polypeptides selected from the group consisting of:

- (i) a polypeptide having a molecular weight of 75 kDa;
- (ii) a polypeptide having a molecular weight of about 77 kDa; and
- 25 (iii) a polypeptide having a molecular weight of about 79 kDa;

wherein each polypeptide has the amino-terminal amino acid sequence  
EDDGFYTSVGYQIGEEAQMV (SEQ ID No.: 7)

52. A method of inducing an immune response in a mammal which comprises administering to said mammal an immunologically effective amount of an antibody by which binds to a polypeptide of Claim 1.

5 53. The method of claim 14 wherein the polypeptide of the antigenic composition is recombinantly produced.

54. A method for producing a desired *H. pylori* protein comprising: (a) transforming a selected host cell with at least one high copy number plasmid, which  
10 comprises the *H. pylori* nucleotide sequence of interest operably linked to a strong promoter, and (b) growing the transformed host cell in culture media.

55. A method of purifying recombinant *H. pylori* polypeptides which are expressed as inclusion bodies in host cells comprising (a) lysing the host cells and  
15 isolating the inclusion bodies by removing soluble proteins; (b) solubilizing the inclusion bodies in a zwitterionic detergent; and (c) purifying the solubilized inclusion bodies.

56. The method of claim wherein at step (c) the solubilized inclusion  
20 body material is purified using a cationic exchange gel chromatograph.

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|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                                                           |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|
| <b>(51) International Patent Classification <sup>6</sup> :</b><br><b>C12N 15/31, 1/21, C07K 14/205, 16/12, A61K 38/16, 39/40 // C07K 14/34, C12N 15/62, A61K 39/106</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |  | <b>A2</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | <b>(11) International Publication Number:</b> <b>WO 00/00614</b><br><b>(43) International Publication Date:</b> 6 January 2000 (06.01.00) |
| <b>(21) International Application Number:</b> PCT/US99/14375<br><b>(22) International Filing Date:</b> 25 June 1999 (25.06.99)<br><b>(30) Priority Data:</b><br>60/090,851 26 June 1998 (26.06.98) US<br><b>(71) Applicant (for all designated States except US):</b> AMERICAN CYANAMID COMPANY [US/US]; Five Giralda Farms, Madison, NJ 07940 (US).<br><b>(72) Inventors; and</b><br><b>(75) Inventors/Applicants (for US only):</b> FULGINITI, James, Peter [US/US]; 5180 Foster Road, Canadagua, NY 14424 (US). FISKE, Michael, James [US/US]; 167 Wood Run, Rochester, NY 14612 (US). DILTS, Deborah, Ann [US/US]; 112 Country Downs Circle, Fairport, NY 14450 (US).<br><b>(74) Agents:</b> WEBSTER, Darryl, L. et al.; American Home Products Corporation, Patent Law Dept. - 2B2, One Campus Drive, Parsippany, NJ 07054 (US). |  | <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).<br><br><b>Published</b><br><i>Without international search report and to be republished upon receipt of that report.</i> |                                                                                                                                           |
| <b>(54) Title:</b> NOVEL ANTIGENS OF <i>HELICOBACTER PYLORI</i><br><br><b>(57) Abstract</b><br><br>The present invention relates to novel nucleic acids and polypeptides relating to <i>Helicobacter pylori</i> , in particular novel <i>H. pylori</i> bacterial surface proteins having molecular weights of approximately 75, 77, and 79 kilo daltons (kDa). The nucleic acid sequences and polypeptides are useful for diagnostic and therapeutic purposes.                                                                                                                                                                                                                                                                                                                                                                        |  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                                                           |



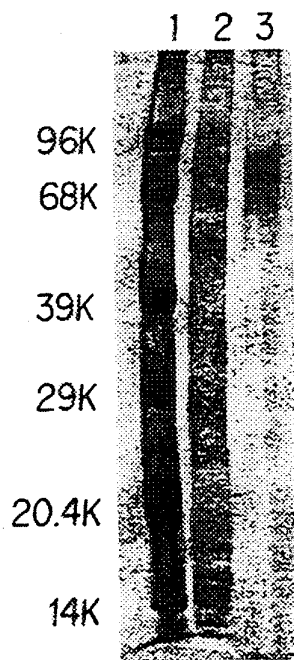


FIG. 1A

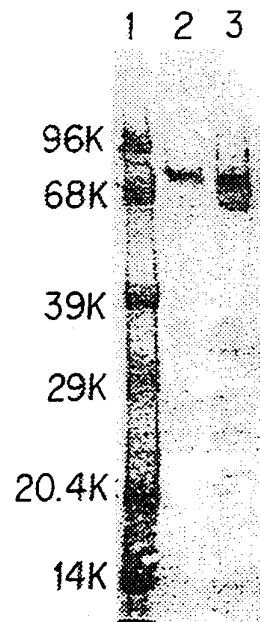


FIG. 1B

2/13



FIG.2

3/13

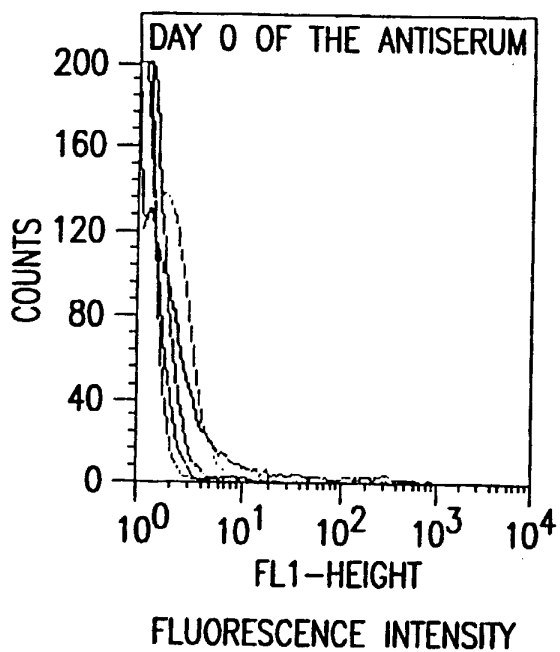


FIG.3A

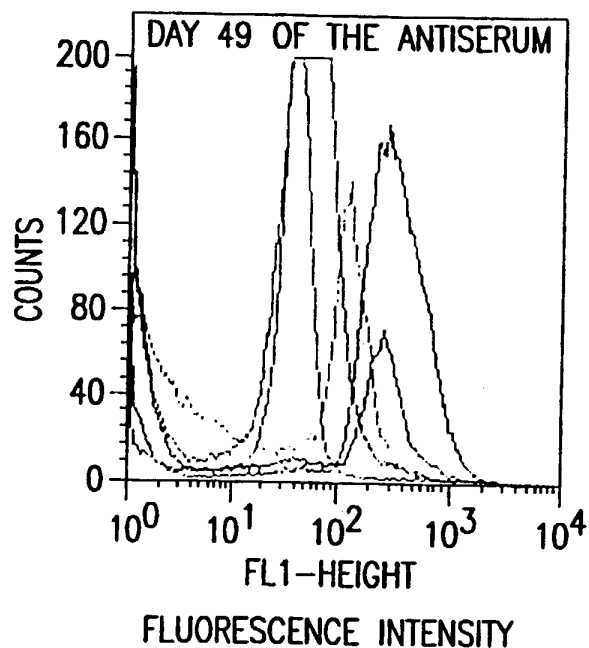


FIG.3B

4/13

09/720560-122100  
09/720560  
PCT/US99/14375

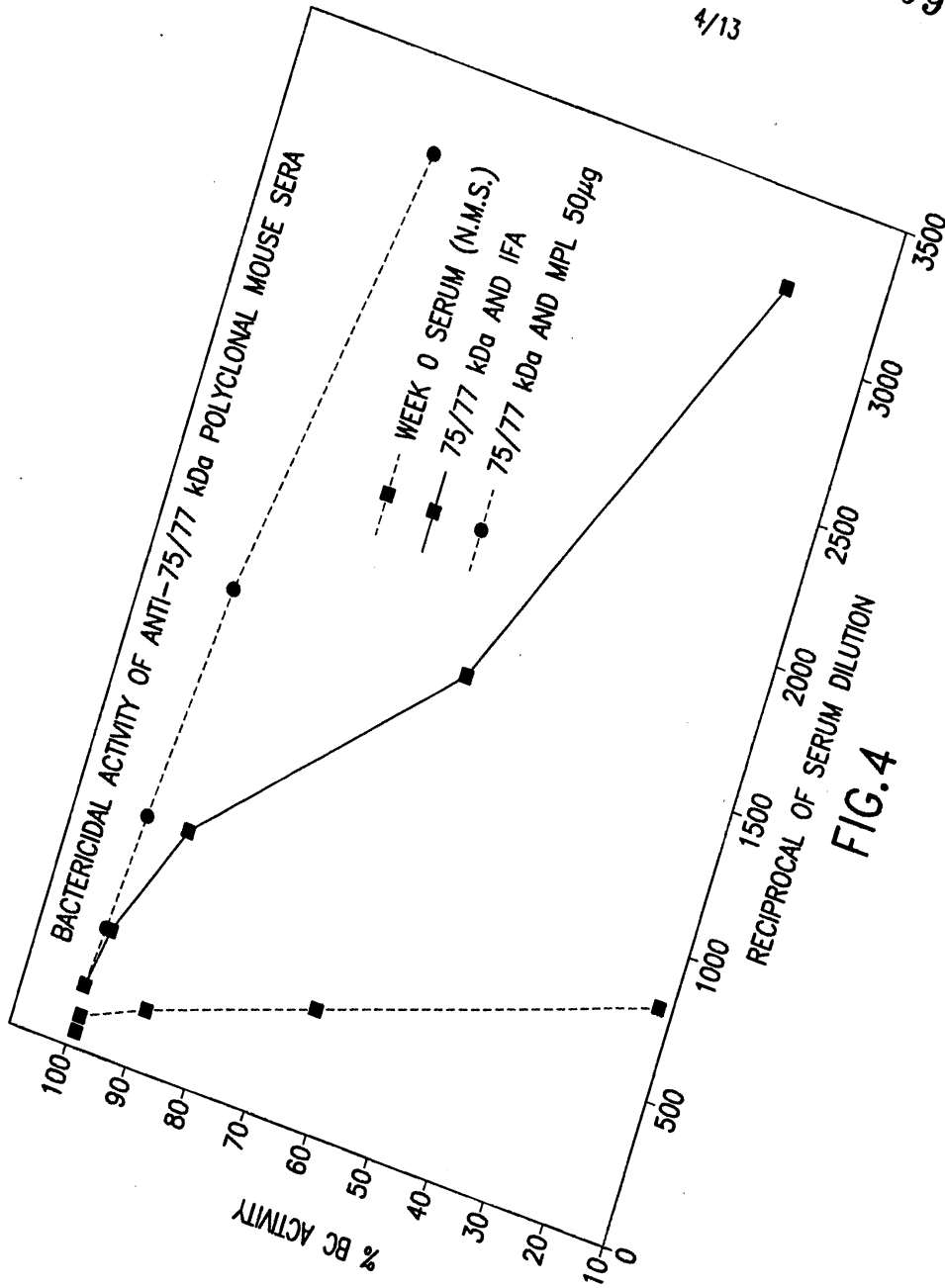


FIG. 4

5/13

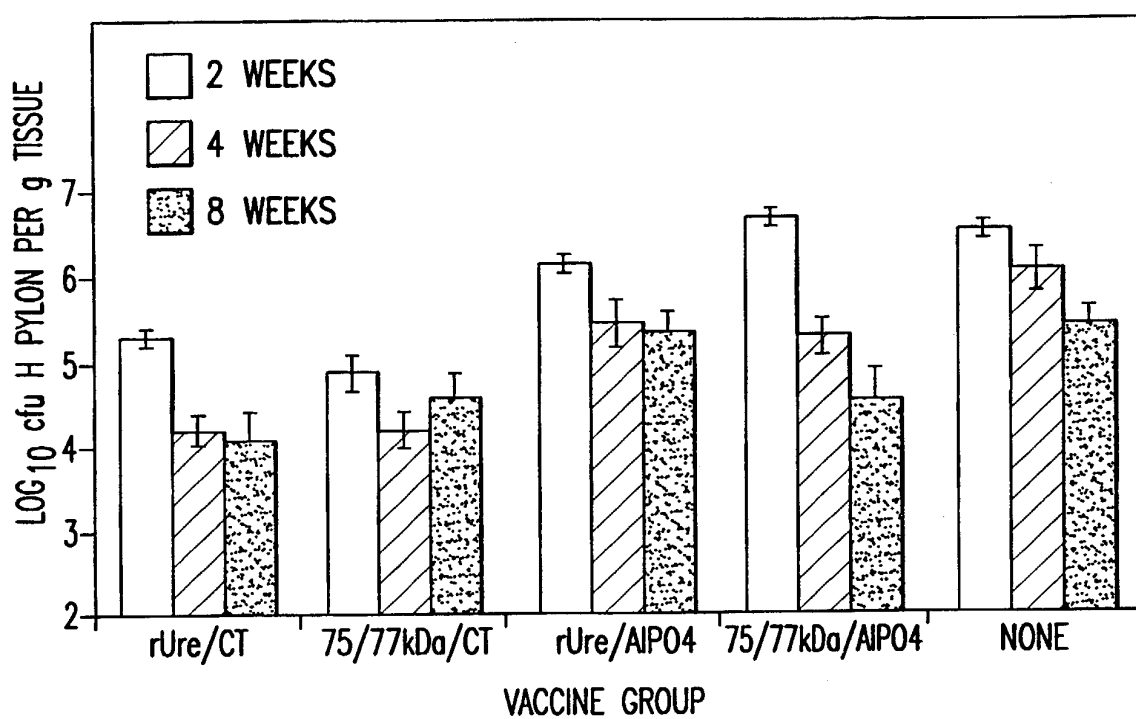


FIG.5

6/13

GAAGACGACGGCTTTTACACAAGCGTAGGCTATCAAATCGGTGAAGCCGCTCAAAT  
GGTAAAAACACCAAAGGCATCCAAGAGCTTTTCAGACAATTATGAAAAGCTGAACAA  
TCTTTTGAATAATTACAGCACCCCTAAACACCCTGATCAAATTATCCGCTGATCCGAG  
CGCAATTAACGACGCAAGGGATAATCTAGGCTCAAGCTCTAGGAATTTGCTTGATGT  
CAAAACCAATTCCCCGGCGTATCAAGCGGTGCTTTTAGCACTGAATGCTGCAGTGG  
GGTTGTGGCAAGTTACAAGCTATGCTTTTACTGCTTGTGGTCCTGGCAGTAACGAG  
AACGCGAATGGAGGTATCCAACTTTTAATAATGTGCCAGGACAAAATACGACGAC  
CATCACTTGCAATTCGTATTATGAGCCAGGACATGGCGGGCCTATATCCACTGCAA  
ACTATGCGAAAATCAATCAAGCCTATCAAATCATTCAAAAGGCTTTGACAGCCAATG  
GAGCTAATGGAGATGGGGTCCCTGTTTTAAGCAACACCACTACAAAACCTTGATTTCA  
CTATCAATGGAGACAAAAGAACGGGGGGCAAACCAAATAAACCATTAAATATACCAAT  
GGAGTCATGGGAAAGCTATTTCAACCTCGTGGAATGACACACAAGCAACACCAACA  
ACAGAAAACATCAACACAGAAAATAACGCTCAAGAGCTTTTAAAGCAAGCGAGCATC  
ATTATCACTACCCTAAATGAGGCATGCCCAAACCTTTCAGAATGGTGGTAGCGGTTAT  
TGCCAAGGGATAAGTGGCAATGGGACAATGTGTGGGATGTTTAAAGATGAAATCAG  
TGCTATCCAAGGCATGATCGCTAACGCGCAAGAAGCTGTGCGCGAAAGTAAAATCG  
TTAGTGAAAACGCGCAAATCAAAACAACCTTGACACTGGAAAACCATTCACCCCT  
ACACAGACGCCAGCTTCGCTCAAAGCATGCTCAAAAACGCTCAAGCCCAAGCAGAG  
ATTTTAAACCAAGCCGAACAAGTGGTGAAAACCTTTGAAAAATCCCTAGAAATTTTG  
TATCAGACTCTTTAGGGGTGTGTTATGAAGTGCAAGGGGGTGAGCGTAGGGGCAC  
CAATCCAGGTCAGGTAACCTTCTAACACTTGGGGGGCCGGTTGCGCCTATGTGGGG  
CAAACCATAACAAATCTAAAAACAGCATCGCTCACTTTGGCACTCAAGAGCAGCAA  
ATACAACGAGCCGAAAATATCGCTGACACTCTAGTGAATTTCAAATCTAGATACAGC  
GAATTGGGGAATACTTACAACAGCATCACCCTGCGCTCTCCAAAGTCCCTAACGC  
GCAAAGCTTGCAAACGTTGGTGAGCAAAAAGAATAACCCCTATAGCCCGCAAGGCA  
TAGAAACCAATTACTACTTGAATCAAATCTTACAACCAAATCCAAACCATCAACCA  
AGAATTAGGGCGTAACCCTTTTAGGAAAGTGGGCATCGTCGGCTCTCAAACCAACA  
ACGGCGCCATGAATGGGATCGGTATTCAGGTGGGCTACAAGCAATTCTTTGGCCAA  
AAAAGAAAATGGGGCGCTAGGTATTACGGCTTTTTTGATTACAACCATGCGTTTATT  
AAATCCAGCTTCTTCAACTCGGCTTCTGATGTGTGGACTTATGGTTTTGGAGCGGAC  
GCTCTCTATAACTTCATCAACGATAAAGCCACTAACTTTTTAGGCAAAAACAACAAG  
CTTTCTGTGGGGCTTTTTGGCGGGATTGCGTTAGCGGGCACTTCATGGCTTAATTC  
TGAGTATGTGAATTTAGCCACCGTGAATAATGTCTATAACGCTAAAATGAACGTGGC  
GAACTTCCAATTCATTCAACATGGGAGTGAGGATGAATTTGGCCAGGCCCAAGA  
AAAAGACAGCGATCATGCGGCTCAGCATGGGATTGAGTTAGGGCTTAAAATCCCC  
ACCATCAACACGAATACTATTCTTTATGGGGGCTGAACTCAAATACAGAAGGCTT  
TATAGCGTGTATTTGAATTATGTGTTCCGCTTACTAA

FIG.6

7/13

EDDGFYTSVGYQIGEEAAQMVKNKTKGIQELSDNYEKLNNLLNNYSTLNTLIKLSADPSA IN  
 DARDNLGSSSRNLLDVKTNSPAYQAVLLALNAAVGLWQVTSYAF TACGPGCSNENANG  
 GIQTFNNVPGQNTTTITCNSYYEPGHGGPISTANYAKINQAYQIIQKALTANGANGDGV  
 VLSNTTTKLDFTINGDKRTGGKPNKPLIYQWSHGKAISTSWNDTQATPTTENINTENNA  
 QELLKQASIIITTLNEACP NFQNGGSGYWQGISGNGTMCGMFKNEISAIQGMIANAE  
 VAQSKIVSENAQNQNNLDTGKPFNPYTDASF AQSM LKNAQAQAEILNQAEQVVKNFEKI  
 PRNFVSDSLGVCYE VQGGERRGTNPGQVTSNTWGAGCAYVGQITNLKNSIAHFGTQ  
 EQQIQRAENIADTLVNFKSRYSELGNTYNSITTALSKVPNAQSLQNVVSKKNNPYS  
 PQG IETNYLLNQNSYNQIQ TINQELGRNPF RKVGI VGSQTNGAMNGIGIQVGYKQFFGQKR  
 KWGARYYGFFDYNHAF IKSSFFNSASDVWTYGF GADALYNF INDKATNFLGKNNKLSV  
 GLFGGIALAGTSWLNSEYVNLATVNNVYNAKMNVANFQFLFMGVRMNLARPKKKDS  
 DHAAQHGI ELGLKIPTINTNYYSFMGAELKYRRLYSVYLYNFAY

FIG.7

EDDGFYTSVGYQIGEEAAQMTNTKGIQELSDNYEKLNNLLNSYSTLNTLIKLSADPSAVS  
 GA INNLNAGATGLLKEKTNSPAYQAVSLALNAAVGLWNTIGYAVMCGNGNGTSNGPGS  
 VIFNGEPGQGSTQITCNRFESTGPGHSM S IDEFKKLNQAYQIIQQALKGNGFPELGNG  
 TSVSVEYKYECKQNNNDIDGGVRQFCQAKNGSSSGSNGSSGNSKQTTTQDGVITTT  
 NSSNEATVNF S ITNNAEQLLNQAANIMQVLNTQCPLVRSTNDENAPGGGQPWGLSTTG  
 NACQIFQQEF SQVTSMIKNAQEI VAQSQIANANQKAEITNPSNFPF TDAGFAQNMLKN  
 ARAQAEMFNLAEQVKKNLEVMQNNNNVNAELSGFGDGMTNFVSAFLASCKNDGTLPN  
 QGVTSNTWGAGCAYVGETITALLNSIAHFGTQAEQIEQAENIADTLVHFKSRYNELGNT  
 YNSITTALSKVPNAQSLQNVMGKKNNPYS PQG IETNYLLNQNSYNQIQ TINQELGRNPF  
 RKVGI VSSQTNGAMNGIGIQVGYKQFFGQKRKWGARYYGFFDYNHAF IKSSFFNSAS  
 DVWTYGF GVDALYNF INDKATNFLGKNNKLSVGLFGGIALAGTSWLNSEYVNLATMNN  
 VYNAKMDVANFQFLFMGVRMNLARPKKKGSDHAAQHGI ELGLKIPTINTNYYSFMGA  
 ELKYRRLYSVYLYNFAY

FIG.9

8/13

GAAGACGACGGCTTTTACACAAGCGTAGGCTATCAAATCGGTAAGCCGCTCAAAT  
GGTAACAAACACCAAAGGCATCCAAGAGCTTTCAGACAATTATGAAAAGCTGAACAA  
TCTTTTGAATAGTTACAGCACCTAAACACCCTTATCAAATTGTCCGCTGATCCGAG  
CGCGGTCACTGGCGCGATCAACAATTTGAACGCGGGCGGACGGGTTTGCTCAAA  
GAAAAAACCAACTCCCCCGCCTATCAAGCCGTCTCTTTGGCGTTAAACGCGCGCAGT  
GGGCTTGTGGAATACCATCGGCTATGCGGTCACTGTGCGGAACGGGAACGGCACA  
TCGAATGGCCCTGGCAGCGTGATCTTTAATGGAGAGCCAGGACAGGGCTCCACGC  
AGATTACTTGCAACCGCTTTGAATCAACTGGTCCTGGTCACAGCATGTCTATTGATG  
AATTCAGAACTCAATCAAGCCTATCAAATCATCCAACAAGCTTTAAAAGGAAATG  
GGTTTCCTGAACTAGGCAACAACGGCACAAGCGTGAGTGTTGAATATAAATATGAAT  
GCAAACAGAATAATAATGATATCGACGGCGGTGTGAGACAATTCTGCCAAGCGAAA  
AATGGTAGTAGCAGTGGTAGTAATGGCAGTAGTGGTAATAGCAAGCAAACAACCAC  
GCAAGACGGCGTAACGATCACCCTACTAATAGTAGTAACGAAGCCACCGTCAATT  
TTAGCATCACCAATAACGCTGAACAGCTGTTAAATCAAGCGGCAAACATCATGCAAG  
TCCTTAACACGCAATGCCCTTTGGTGCGATCTACGAATGATGAAAACGCTCCAGGG  
GGTGCCAACCATGGGGTTTAAGCACGACAGGGAATGCGTGCCAAATCTTCCAACA  
AGAATTTAGCCAGGTTACTAGCATGATCAAAAACGCCCAAGAAATCGTCGCGCAAA  
GCCAAATCGCTAACGCTAACCAAAAAGCAGAAATAACTAACCCAGTAACCTTCAACC  
CTTTCACGGACGCTGGCTTTGCACAAAACATGCTCAAAAACGCTAGAGCGCAAGCA  
GAGATGTTCAATTTAGCTGAGCAAGTGAAAAAGAATTTGGAAGTCATGCAAAACAAC  
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GCCTTTTGGCAAGCTGCAAAAATGATGGGACATTGCCTAATCAAGGGTTACTTCT  
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GGGAAAAAAGAATAACCCCTATAGCCACAAGGCATAGAAACCAATTACTATCTCAA  
TCAAACTCTTACAACCAATCCAACCATCAACCAAGAATTAGGCCGTAAACCCCTT  
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ATGGGAGTGAGGATGAATTTAGCCAGACCCAAGAAAAAAGGCAGCGATCATGCGG  
CTCAGCATGGGATTGAGTTAGGGCTTAAAATCCCCACCATCAACACGAACTACTATT  
CCTTTATGGGGGCTGAACCTAAATACAGAAGGCTCTATAGCGTGATTTGAATTATG  
TGTTGCTTACTAA

FIG.8



9/13

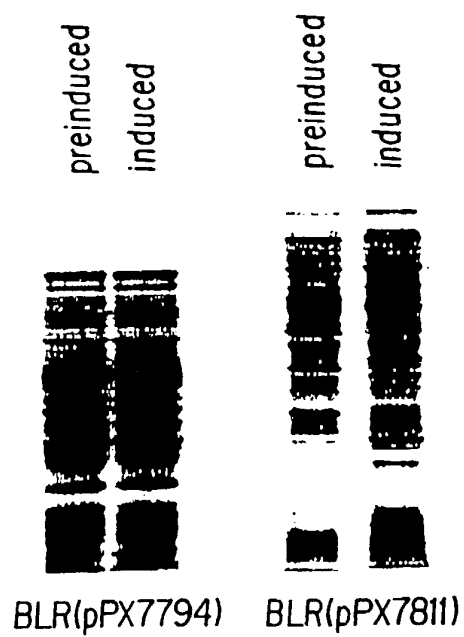


FIG.10

10/13

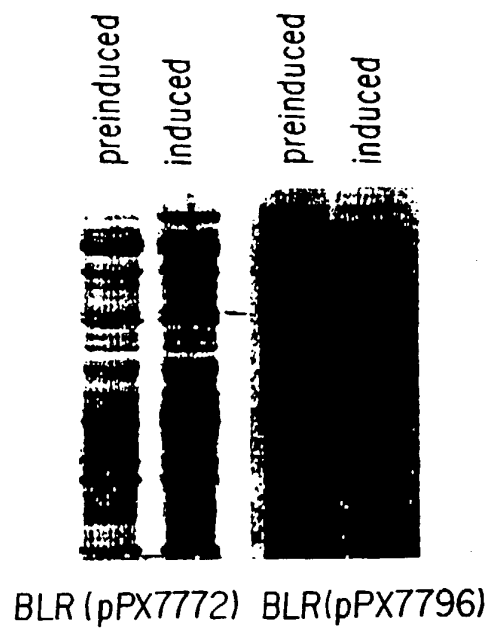


FIG.11

11/13

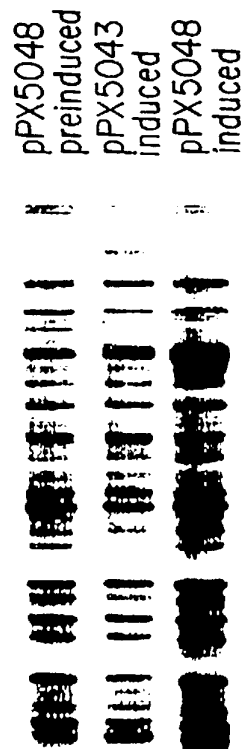


FIG.12

12/13

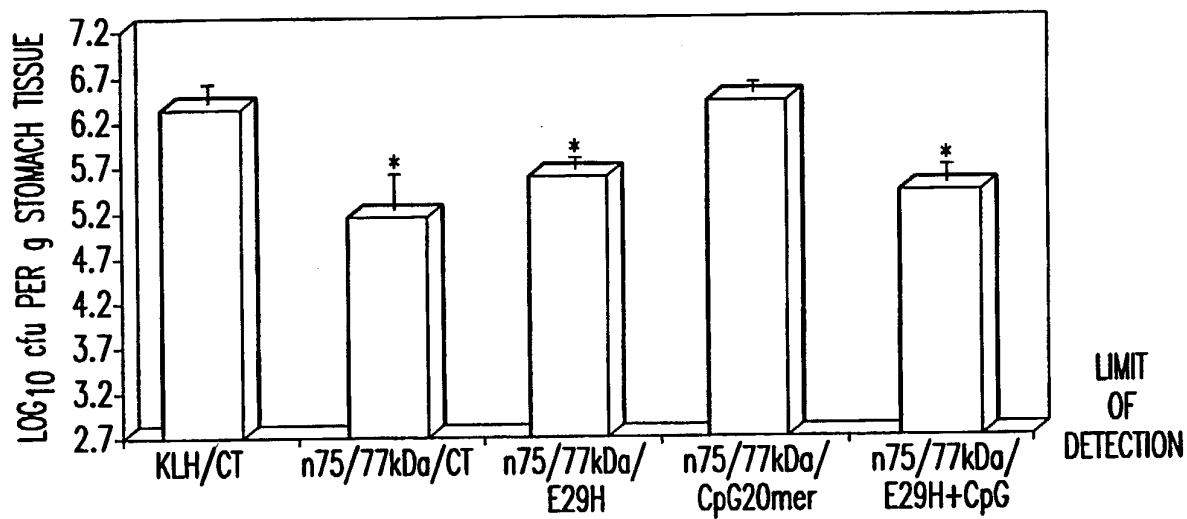


FIG.13

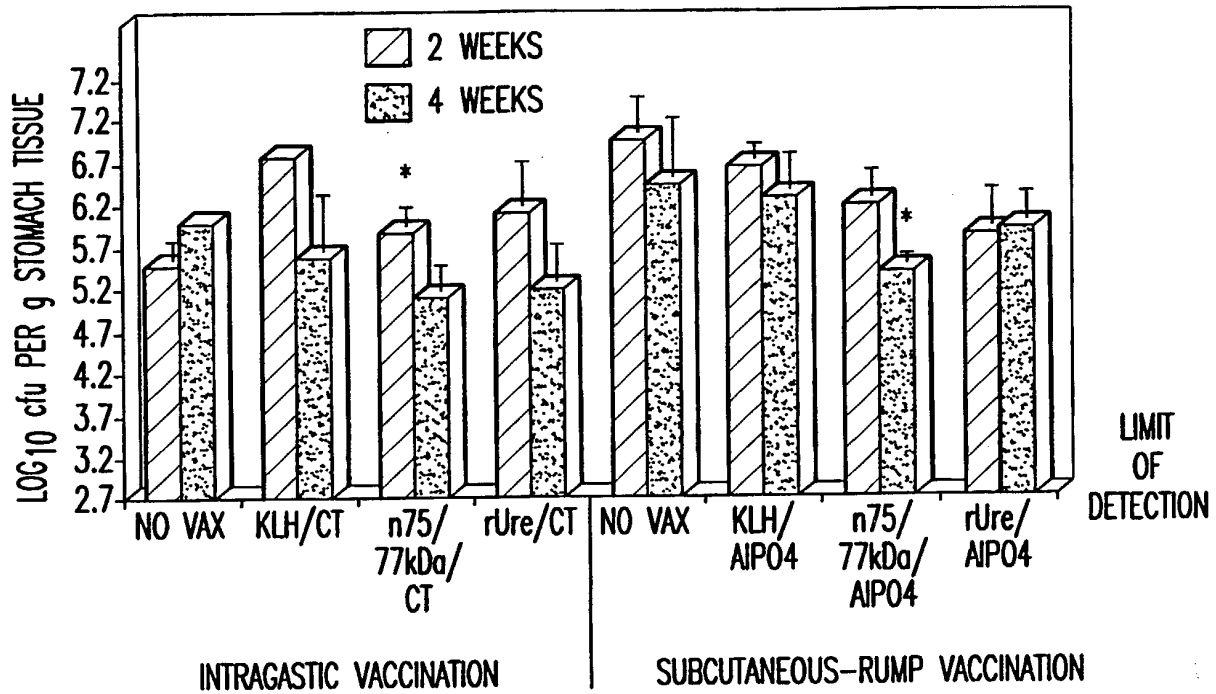


FIG.14

COMBINED DECLARATION AND POWER OF ATTORNEY  
(Original, Design, Supplemental, Divisional, Continuation, CIP)

As the below named inventor, I hereby declare that:

INVENTORSHIP IDENTIFICATION

My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

TITLE OF INVENTION

NOVEL ANTIGENS OF HELICOBACTER PYLORI

SPECIFICATION IDENTIFICATION

the specification of which: (complete (a), (b), or (c))

- (a) ☐ is attached hereto.
- (b) ☐ was filed on \_\_\_\_\_ as  
☐ Serial Number  
☐ Express Mail No. \_\_\_\_\_, as Serial Number not yet known
- (c) ☒ was described and claimed in PCT International Application No.  
PCT/US99/14375 filed on June 25, 1999 and as amended under PCT Article  
19 on \_\_\_\_\_ (if any).

ACKNOWLEDGEMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37 CFR 1.56(a).

PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventors certificate or of any PCT International application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate of any PCT International application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

- (d) ☒ No such applications have been filed.  
(e) ☐ Such applications have been filed as follows.

NOTE: Where item (c) is entered above and the International Application which designated the U.S. claimed priority, check item (e), enter the details below and make the priority claim.

Earliest Foreign Application(s), if any, filed within 12 months (6 months for Design) prior to this U.S. Application

| Country | Application No. | Date of Filing<br>(Day, Month, Year) | Priority Claimed<br>35 USC 119 |
|---------|-----------------|--------------------------------------|--------------------------------|
|         |                 |                                      |                                |
|         |                 |                                      |                                |
|         |                 |                                      |                                |
|         |                 |                                      |                                |

All Foreign Application(s), if any, Filed More Than 12 Months  
(6 Months for Design) Prior to This U.S. Application

**CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)**  
(35 U.S.C. § 119(E))

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

**PROVISIONAL APPLICATION NUMBER**

**FILING DATE**

60/090,851

June 26, 1998

**CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)**  
(UNDER 35 U.S.C. 120)

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT International filing date of this application.

**PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS**  
**DESIGNATING THE U.S. FOR BENEFIT UNDER 35 USC 120**

| U.S. Applications |                  | Status (Check One)       |                          |                          |
|-------------------|------------------|--------------------------|--------------------------|--------------------------|
| U.S. Applications | U.S. Filing Date | Patented                 | Pending                  | Abandoned                |
|                   |                  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                   |                  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                   |                  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                   |                  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |



| PCT Applications Designating U.S. |                 |                                      |
|-----------------------------------|-----------------|--------------------------------------|
| PCT APPLICATION NO.               | PCT FILING DATE | U.S. SERIAL NO. ASSIGNED<br>(if any) |
| PCT/US99/14375                    | June 25, 1999   |                                      |
|                                   |                 |                                      |

POWER OF ATTORNEY

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

23 Elizabeth M. Barnhard, Reg. No. 31,088; Rebecca R. Barrett, Reg. No. 35,152; Egon E. Berg, Reg. No. 21,117; William H. Calnan, Reg. No. 29,520; Milagros A. Cepeda, Reg. No. 33,365; Steven R. Eck, Reg. No. 36,126; Bruce M. Eisen, Reg. No. 22,847; Steven H. Flynn, Reg. No. 29,639; Alan M. Gordon, Reg. No. 30,637; Barbara A. Gyure, Reg. No. 34,614; John W. Hogan, Jr., Reg. No. 32,703; Elizabeth A. Hurley, Reg. No. 41,859; Adley F. Mandel, Reg. No. 26,942; Gale F. Matthews, Reg. No. 32,269; Joseph M. Mazzaresse, Reg. No. 32,803; Arnold S. Milowsky, Reg. No. 35,288; Daniel B. Moran, Reg. No. 41,204; Michael R. Nagy, Reg. No. 33,432; Barbara L. Renda, Reg. No. 27,626; M. Andrea Ryan, Reg. No. 28,469; Thomas S. Szatkowski, Reg. No. 28,049; George Tarnowski, Reg. No. 27,472; and Darryl L. Webster, Reg. No. 34,276 all of AMERICAN HOME PRODUCTS CORPORATION, One Campus Drive, Parsippany, New Jersey 07054.

☐ Attached as part of this declaration and power of attorney is the authorization of the above-named attorney(s) to accept and follow instructions from my representative(s).

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Docket No: ACY33427-00  
Patent

## DECLARATION

I hereby declare that all statements herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURE(S)

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Inventor's Signature: James P. Fulpink

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Full name of SECOND JOINT INVENTOR: **Michael James FISKE**

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Full name of FOURTH JOINT INVENTOR:

Inventor's Signature: \_\_\_\_\_

Date: \_\_\_\_\_

Country of Citizenship:

Residence :

Post Office Address:

Rec'd PCT/PTO 01 OCT 2001

#3

ACY33427 PCT/US  
PATENTS

**UNDER THE PATENT COOPERATION TREATY**

Applicant(s): AMERICAN CYANAMID COMPANY Customer Number: 25291  
U.S. Appl'n No. 09/720,560  
Int'l Appl'n No.: PCT/US 99/14375  
Int'l Filing Date: 25 JUNE 1999  
For: Novel Antigens of Helicobacter Pylori

October 1, 2001

**Notification and Request for Change of Address**


Commissioner for Patents  
Box PCT  
United States Patent and Trademark Office  
Washington, DC 20231  
Attn: John L. Anderson

S I R:

Please make of record in this patent application the following  
new correspondence address for all correspondence regarding the above-identified  
National Phase Application:

Darryl L. Webster  
American Home Products Corporation  
Patent law Department  
5 Giralda Farms  
Madison, NJ 07940

Respectfully submitted,  
Fulginiti et al

  
Darryl L. Webster  
Attorney for Applicants  
Reg. No. 34,276

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Patent Law Dept.  
5 Giralda Farms  
Madison, NJ 07940  
Phone: 973-683-2159  
Facsimile: 973-683-2117

WO 00/00614

PCT/US99/14375

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Fulginiti, James P.  
Fiske, Michael J.  
... .Dilts, Deborah A.
- (ii) TITLE OF INVENTION: Novel Antigens of Helicobacter pylori
- (iii) NUMBER OF SEQUENCES: 10
- (iv) CORRESPONDENCE ADDRESS:  
(A) ADDRESSEE: American Cyanamid Company  
(B) STREET: One Campus Drive  
(C) CITY: Parsippany  
(D) STATE: New Jersey  
(E) COUNTRY: USA  
(F) ZIP: 07054
- (v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER: US  
(B) FILING DATE:  
(C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: Webster, Darryl L.  
(B) REGISTRATION NUMBER: 34276  
(C) REFERENCE/DOCKET NUMBER: 33427-00L
- (ix) TELECOMMUNICATION INFORMATION:  
(A) TELEPHONE: 973-683-2159  
(B) TELEFAX: 973-683-4117

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 708 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Lys Lys Thr Leu Leu Leu Ser Leu Ser Leu Ser Phe Leu  
1 5 10 15

WO 00/00614

PCT/US99/14375

Leu His Ala Glu Asp Asp Gly Phe Tyr Thr Ser Val Gly Tyr Gln Ile  
 20 25 30  
 Gly Glu Ala Ala Gln Met Val Lys Asn Thr Lys Gly Ile Gln Glu Leu  
 35 40 45  
 Ser Asp Asn Tyr Glu Lys Leu Asn Asn Leu Leu Asn Asn Tyr Ser Thr  
 50 55 60  
 Leu Asn Thr Leu Ile Lys Leu Ser Ala Asp Pro Ser Ala Ile Asn Asp  
 65 70 75 80  
 Ala Arg Asp Asn Leu Gly Ser Ser Ser Arg Asn Leu Leu Asp Val Lys  
 85 90 95  
 Thr Asn Ser Pro Ala Tyr Gln Ala Val Leu Leu Ala Leu Asn Ala Ala  
 100 105 110  
 Val Gly Leu Trp Gln Val Thr Ser Tyr Ala Phe Thr Ala Cys Gly Pro  
 115 120 125  
 Gly Ser Asn Glu Asn Ala Asn Gly Gly Ile Gln Thr Phe Asn Asn Val  
 130 135 140  
 Pro Gly Gln Asp Thr Thr Thr Ile Thr Cys Asn Ser Tyr Tyr Glu Pro  
 145 150 155 160  
 Gly His Gly Gly Pro Ile Ser Thr Ala Asn Tyr Ala Lys Ile Asn Gln  
 165 170 175  
 Ala Tyr Gln Ile Ile Gln Lys Ala Leu Thr Ala Asn Gly Ala Asn Gly  
 180 185 190  
 Asp Gly Val Pro Val Leu Ser Asn Thr Thr Thr Lys Leu Asp Phe Thr  
 195 200 205  
 Ile Asn Gly Asp Lys Arg Thr Gly Gly Lys Pro Asn Thr Pro Glu Lys  
 210 215 220  
 Phe Pro Trp Ser Asp Gly Lys Tyr Ile His Thr Gln Trp Ile Asn Thr  
 225 230 235 240  
 Ile Val Thr Pro Thr Glu Thr Asn Ile Asn Thr Glu Asn Asn Ala Gln  
 245 250 255  
 Glu Leu Leu Lys Gln Ala Ser Ile Ile Ile Thr Thr Leu Asn Glu Ala  
 260 265 270  
 Cys Pro Asn Phe Gln Asn Gly Gly Arg Ser Tyr Trp Gln Gly Ile Ser  
 275 280 285  
 Gly Asn Gly Thr Met Cys Gly Met Phe Lys Asn Glu Ile Ser Ala Ile  
 290 295 300  
 Gln Gly Met Ile Ala Asn Ala Gln Glu Ala Val Ala Gln Ser Lys Ile  
 305 310 315 320  
 Val Ser Glu Asn Ala Gln Asn Gln Asn Asn Leu Asp Thr Gly Lys Pro

WO 00/00614

PCT/US99/14375

| 325 |     |     |     |     |     |     |     |     |     | 330 |     |     |     |     | 335 |  |  |  |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|--|--|
| Phe | Asn | Pro | Tyr | Thr | Asp | Ala | Ser | Phe | Ala | Gln | Ser | Met | Leu | Lys | Asn |  |  |  |  |
|     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |  |  |  |  |
| Ala | Gln | Ala | Gln | Ala | Glu | Ile | Leu | Asn | Gln | Ala | Glu | Gln | Val | Val | Lys |  |  |  |  |
|     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |  |  |  |  |
| Asn | Phe | Glu | Lys | Ile | Pro | Thr | Ala | Phe | Val | Ser | Asp | Ser | Leu | Gly | Val |  |  |  |  |
|     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |  |  |  |  |
| Cys | Tyr | Glu | Val | Gln | Gly | Gly | Glu | Arg | Arg | Gly | Thr | Asn | Pro | Gly | Gln |  |  |  |  |
| 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     | 400 |  |  |  |  |
| Val | Thr | Ser | Asn | Thr | Trp | Gly | Ala | Gly | Cys | Ala | Tyr | Val | Lys | Gln | Thr |  |  |  |  |
|     |     |     |     | 405 |     |     |     |     | 410 |     |     |     |     | 415 |     |  |  |  |  |
| Ile | Thr | Asn | Leu | Asp | Asn | Ser | Ile | Ala | His | Phe | Gly | Thr | Gln | Glu | Gln |  |  |  |  |
|     |     |     | 420 |     |     |     |     | 425 |     |     |     |     | 430 |     |     |  |  |  |  |
| Gln | Ile | Gln | Gln | Ala | Glu | Asn | Ile | Ala | Asp | Thr | Leu | Val | Asn | Phe | Lys |  |  |  |  |
|     |     | 435 |     |     |     |     | 440 |     |     |     |     | 445 |     |     |     |  |  |  |  |
| Ser | Arg | Tyr | Ser | Glu | Leu | Gly | Asn | Thr | Tyr | Asn | Ser | Ile | Thr | Thr | Ala |  |  |  |  |
|     | 450 |     |     |     |     | 455 |     |     |     |     | 460 |     |     |     |     |  |  |  |  |
| Leu | Ser | Lys | Val | Pro | Asn | Ala | Gln | Ser | Leu | Gln | Asn | Val | Val | Ser | Lys |  |  |  |  |
| 465 |     |     |     |     | 470 |     |     |     |     | 475 |     |     |     |     | 480 |  |  |  |  |
| Lys | Asn | Asn | Pro | Tyr | Ser | Pro | Gln | Gly | Ile | Glu | Thr | Asn | Tyr | Tyr | Leu |  |  |  |  |
|     |     |     |     | 485 |     |     |     |     | 490 |     |     |     |     | 495 |     |  |  |  |  |
| Asn | Gln | Asn | Ser | Tyr | Asn | Gln | Ile | Gln | Thr | Ile | Asn | Gln | Glu | Leu | Gly |  |  |  |  |
|     |     |     | 500 |     |     |     |     | 505 |     |     |     |     | 510 |     |     |  |  |  |  |
| Arg | Asn | Pro | Phe | Arg | Lys | Val | Gly | Ile | Val | Asn | Ser | Gln | Thr | Asn | Asn |  |  |  |  |
|     |     | 515 |     |     |     |     | 520 |     |     |     |     | 525 |     |     |     |  |  |  |  |
| Gly | Ala | Met | Asn | Gly | Ile | Gly | Ile | Gln | Val | Gly | Tyr | Lys | Gln | Phe | Phe |  |  |  |  |
|     | 530 |     |     |     |     | 535 |     |     |     |     | 540 |     |     |     |     |  |  |  |  |
| Gly | Gln | Lys | Arg | Lys | Trp | Gly | Ala | Arg | Tyr | Tyr | Gly | Phe | Phe | Asp | Tyr |  |  |  |  |
| 545 |     |     |     |     | 550 |     |     |     |     | 555 |     |     |     |     | 560 |  |  |  |  |
| Asn | His | Ala | Phe | Ile | Lys | Ser | Ser | Phe | Phe | Asn | Ser | Ala | Ser | Asp | Val |  |  |  |  |
|     |     |     |     | 565 |     |     |     |     | 570 |     |     |     |     | 575 |     |  |  |  |  |
| Trp | Thr | Tyr | Gly | Phe | Gly | Ala | Asp | Ala | Leu | Tyr | Asn | Phe | Ile | Asn | Asp |  |  |  |  |
|     |     |     | 580 |     |     |     |     | 585 |     |     |     |     | 590 |     |     |  |  |  |  |
| Lys | Ala | Thr | Asn | Phe | Leu | Gly | Lys | Asn | Asn | Lys | Leu | Ser | Val | Gly | Leu |  |  |  |  |
|     |     | 595 |     |     |     |     | 600 |     |     |     |     | 605 |     |     |     |  |  |  |  |
| Phe | Gly | Gly | Ile | Ala | Leu | Ala | Gly | Thr | Ser | Trp | Leu | Asn | Ser | Glu | Tyr |  |  |  |  |
|     | 610 |     |     |     |     | 615 |     |     |     |     | 620 |     |     |     |     |  |  |  |  |
| Val | Asn | Leu | Ala | Thr | Val | Asn | Asn | Val | Tyr | Asn | Ala | Lys | Met | Asn | Val |  |  |  |  |
| 625 |     |     |     |     | 630 |     |     |     |     | 635 |     |     |     |     | 640 |  |  |  |  |

WO 00/00614

PCT/US99/14375

Ala Asn Phe Gln Phe Leu Phe Asn Met Gly Val Arg Met Asn Leu Ala  
645 650 655

Arg Ser Lys Lys Lys Gly Ser Asp His Ala Ala Gln His Gly Ile Glu  
660 665 670

Leu Gly Leu Lys Ile Pro Thr Ile Asn Thr Asn Tyr Tyr Ser Phe Met  
675 680 685

Gly Ala Glu Leu Lys Tyr Arg Arg Leu Tyr Ser Val Tyr Leu Asn Tyr  
690 695 700

Val Phe Ala Tyr  
705

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 741 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Lys Lys His Ile Leu Ser Leu Ala Leu Gly Ser Leu Leu Val Ser  
1 5 10 15

Thr Leu Ser Ala Glu Asp Asp Gly Phe Tyr Thr Ser Val Gly Tyr Gln  
20 25 30

Ile Gly Glu Ala Ala Gln Met Val Thr Asn Thr Lys Gly Ile Gln Asp  
35 40 45

Leu Ser Asp Arg Tyr Glu Ser Leu Asn Asn Leu Leu Thr Arg Tyr Ser  
50 55 60

Thr Leu Asn Thr Leu Ile Lys Leu Ser Ala Asp Pro Ser Ala Ile Asn  
65 70 75 80

Ala Ala Arg Glu Asn Leu Gly Ala Ser Ala Lys Asn Leu Ile Gly Asp  
85 90 95

Lys Ala Asn Ser Pro Ala Tyr Gln Ala Val Leu Leu Ala Ile Asn Ala  
100 105 110

Ala Val Gly Phe Trp Asn Val Leu Gly Tyr Ala Thr Gln Cys Gly Gly  
115 120 125

Asn Ala Asn Gly Gln Lys Ser Thr Ser Ser Thr Thr Ile Phe Asn Asn  
130 135 140

Glu Pro Gly Tyr Arg Ser Thr Ser Ile Thr Cys Ser Leu Asn Gly Tyr  
145 150 155 160

WO 00/00614

PCT/US99/14375

Thr Pro Gly Tyr Tyr Gly Pro Met Ser Ile Glu Asn Phe Lys Lys Leu  
 165 170 175  
 Asn Glu Ala Tyr Gln Ile Leu Gln Thr Ala Leu Lys Gln Gly Leu Pro  
 180 185 190  
 Ala Leu Lys Glu Asn Asn Lys Lys Val Asn Val Thr Tyr Thr Tyr Thr  
 195 200 205  
 Cys Ser Gly Gly Gly Asn Asn Asn Cys Ser Ser Glu Ala Thr Gly Val  
 210 215 220  
 Ser Asn Gln Asn Gly Gly Thr Lys Thr Thr Thr Gln Thr Ile Asp Gly  
 225 230 235 240  
 Lys Ser Val Thr Thr Thr Ile Ser Ser Lys Val Val Asp Ser Thr Ala  
 245 250 255  
 Ser Gly Asn Thr Ser Arg Val Ser Tyr Thr Glu Ile Thr Asn Lys Leu  
 260 265 270  
 Glu Gly Val Pro Asp Ser Ala Gln Ala Leu Leu Ala Gln Ala Ser Thr  
 275 280 285  
 Leu Ile Ser Thr Ile Asn Thr Ala Cys Pro Phe Phe Ser Val Thr Asn  
 290 295 300  
 Gln Ser Gly Gly Pro Gln Met Glu Pro Thr Lys Gly Lys Leu Cys Gly  
 305 310 315 320  
 Phe Thr Glu Glu Ile Ser Ala Ile Gln Lys Met Ile Thr Asp Ala Gln  
 325 330 335  
 Glu Leu Val Asn Gln Thr Ser Val Ile Asn Ser His Glu Gln Ser Thr  
 340 345 350  
 Leu Val Gly Gly Asn Asn Gly Lys Pro Phe Asn Pro Phe Thr Asp Ala  
 355 360 365  
 Gln Phe Ala Gln Gly Met Leu Ala Asn Ala Ser Ala Gln Ala Lys Met  
 370 375 380  
 Leu Asn Leu Ala His Gln Val Gly Gln Thr Ile Asn Pro Asn Asn Leu  
 385 390 395 400  
 Thr Gly Asn Phe Lys Asn Phe Val Thr Gly Phe Leu Ala Thr Cys Asn  
 405 410 415  
 Asn Pro Ser Thr Ala Gly Thr Gly Gly Thr Gln Gly Ser Ala Pro Gly  
 420 425 430  
 Thr Val Thr Thr Gln Thr Phe Ala Ser Gly Cys Ala Tyr Val Glu Gln  
 435 440 445  
 Thr Ile Thr Asn Leu Glu Asn Ser Ile Ala His Phe Gly Thr Gln Glu  
 450 455 460  
 Gln Gln Ile Gln Arg Ala Glu Asn Ile Ala Asp Thr Leu Val Asn Phe



WO 00/00614

PCT/US99/14375

|                                                                 |                                                 |     |  |     |  |     |
|-----------------------------------------------------------------|-------------------------------------------------|-----|--|-----|--|-----|
| 465                                                             |                                                 | 470 |  | 475 |  | 480 |
| Lys Ser Arg Tyr                                                 | Ser Glu Leu Gly Asn Thr Tyr Asn Ser Ile Thr Thr |     |  |     |  |     |
|                                                                 | 485                                             |     |  | 490 |  | 495 |
| Ala Leu Ser Lys                                                 | Val Pro Asn Ala Gln Ser Leu Gln Asn Val Val Ser |     |  |     |  |     |
|                                                                 | 500                                             |     |  | 505 |  | 510 |
| Lys Lys Asn Asn Pro Tyr Ser                                     | Pro Gln Gly Ile Glu Thr Asn Tyr Tyr             |     |  |     |  |     |
|                                                                 | 515                                             |     |  | 520 |  | 525 |
| Leu Asn Gln Asn Ser Tyr Asn Gln Ile Gln Thr Ile Asn Gln Glu Leu |                                                 |     |  |     |  |     |
|                                                                 | 530                                             |     |  | 535 |  | 540 |
| Gly Arg Asn Pro Phe Arg Lys Val Gly Ile Val Gly Ser Gln Thr Asn |                                                 |     |  |     |  |     |
|                                                                 | 545                                             |     |  | 550 |  | 555 |
|                                                                 |                                                 |     |  |     |  | 560 |
| Asn Gly Ala Met Asn Gly Ile Gly Ile Gln Val Gly Tyr Glu Gln Phe |                                                 |     |  |     |  |     |
|                                                                 | 565                                             |     |  | 570 |  | 575 |
| Phe Gly Gln Lys Arg Lys Trp Gly Ala Arg Tyr Tyr Gly Phe Phe Asp |                                                 |     |  |     |  |     |
|                                                                 | 580                                             |     |  | 585 |  | 590 |
| Tyr Asn His Ala Phe Ile Lys Ser Ser Phe Phe Asn Ser Ala Ser Asp |                                                 |     |  |     |  |     |
|                                                                 | 595                                             |     |  | 600 |  | 605 |
| Val Trp Thr Tyr Gly Phe Gly Ala Asp Ala Leu Tyr Asn Phe Ile Asn |                                                 |     |  |     |  |     |
|                                                                 | 610                                             |     |  | 615 |  | 620 |
| Asp Lys Ala Thr Asn Phe Leu Gly Lys Asn Asn Lys Leu Ser Val Gly |                                                 |     |  |     |  |     |
|                                                                 | 625                                             |     |  | 630 |  | 635 |
| Leu Phe Gly Gly Ile Ala Leu Ala Gly Thr Ser Trp Leu Asn Ser Glu |                                                 |     |  |     |  |     |
|                                                                 | 645                                             |     |  | 650 |  | 655 |
| Tyr Val Asn Leu Ala Thr Val Asn Asn Val Tyr Asn Ala Lys Met Asn |                                                 |     |  |     |  |     |
|                                                                 | 660                                             |     |  | 665 |  | 670 |
| Val Ala Asn Phe Gln Phe Leu Phe Asn Met Gly Val Arg Met Asn Leu |                                                 |     |  |     |  |     |
|                                                                 | 675                                             |     |  | 680 |  | 685 |
| Ala Arg Pro Lys Lys Asn Asp Ser Asp His Ala Ala Gln His Gly Ile |                                                 |     |  |     |  |     |
|                                                                 | 690                                             |     |  | 695 |  | 700 |
| Glu Leu Gly Leu Lys Ile Pro Thr Ile Asn Thr Asn Tyr Tyr Ser Phe |                                                 |     |  |     |  |     |
|                                                                 | 705                                             |     |  | 710 |  | 715 |
| Met Gly Ala Glu Leu Lys Tyr Arg Arg Leu Tyr Ser Val Tyr Leu Asn |                                                 |     |  |     |  |     |
|                                                                 | 725                                             |     |  | 730 |  | 735 |
| Tyr Val Phe Ala Tyr                                             |                                                 |     |  |     |  |     |
|                                                                 | 740                                             |     |  |     |  |     |

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 745 amino acids

(B) TYPE: amino acid

WO 00/00614

PCT/US99/14375

(C) STRANDEDNESS:  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

Met Lys Lys His Ile Leu Ser Leu Ala Leu Gly Ser Leu Leu Val Ser
1      5      10      15
Thr Leu Ser Ala Glu Asp Asp Gly Phe Tyr Thr Ser Val Gly Tyr Gln
20      25      30
Ile Gly Glu Ala Ala Gln Met Val Thr Asn Thr Lys Gly Ile Gln Gln
35      40      45
Leu Ser Asp Asn Tyr Glu Asn Leu Asn Asn Leu Leu Thr Arg Tyr Ser
50      55      60
Thr Leu Asn Thr Leu Ile Lys Leu Ser Ala Asp Pro Ser Ala Ile Asn
65      70      75      80
Ala Val Arg Glu Asn Leu Gly Ala Ser Thr Lys Asn Leu Ile Gly Asp
85      90      95
Lys Ala Asn Ser Pro Ala Tyr Gln Ala Val Phe Leu Ala Ile Asn Ala
100     105     110
Ala Val Gly Leu Trp Asn Thr Ile Gly Tyr Ala Val Met Cys Gly Asn
115     120     125
Gly Asn Gly Thr Glu Ser Gly Pro Gly Ser Val Ile Phe Asn Asp Gln
130     135     140
Pro Gly Gln Asp Ser Thr Gln Ile Thr Cys Asn Arg Phe Glu Ser Thr
145     150     155     160
Gly Pro Gly Lys Ser Met Ser Ile Asp Glu Phe Lys Lys Leu Asn Glu
165     170     175
Ala Tyr Gln Ile Ile Gln Gln Ala Leu Lys Asn Gln Ser Gly Phe Pro
180     185     190
Glu Leu Gly Gly Asn Gly Thr Lys Val Ser Val Asn Tyr Asn Tyr Glu
195     200     205
Cys Arg Gln Thr Ala Asp Ile Asn Gly Gly Val Tyr Gln Phe Cys Lys
210     215     220
Ala Lys Asn Gly Ser Ser Ser Ser Ser Asn Gly Gly Asn Gly Ser Ser
225     230     235     240
Thr Gln Thr Thr Ala Thr Thr Thr Gln Asp Gly Val Thr Ile Thr Thr
245     250     255
Thr Tyr Asn Asn Asn Lys Ala Thr Val Lys Phe Asp Ile Thr Asn Asn
260     265     270

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WO 00/00614

PCT/US99/14375

Ala Glu Gln Leu Leu Asn Gln Ala Ala Asn Ile Met Gln Val Leu Asn  
 275 280 285  
 Thr Gln Cys Pro Leu Val Arg Ser Thr Asn Asn Glu Asn Thr Pro Gly  
 290 295 300  
 Gly Gly Gln Pro Trp Gly Leu Ser Thr Ser Gly Asn Ala Cys Ser Ile  
 305 310 315 320  
 Phe Gln Gln Glu Phe Ser Gln Val Thr Ser Met Ile Lys Asn Ala Gln  
 325 330 335  
 Glu Ile Ile Ala Gln Ser Lys Ile Val Ser Glu Asn Ala Gln Asn Gln  
 340 345 350  
 Asn Asn Leu Asp Thr Gly Lys Pro Phe Asn Pro Tyr Thr Asp Ala Ser  
 355 360 365  
 Phe Ala Gln Ser Met Leu Lys Asn Ala Gln Ala Gln Ala Glu Met Phe  
 370 375 380  
 Asn Leu Ser Glu Gln Val Lys Lys Asn Leu Glu Val Met Lys Asn Asn  
 385 390 395 400  
 Asn Asn Val Asn Glu Lys Leu Ala Gly Phe Gly Lys Glu Glu Val Met  
 405 410 415  
 Thr Asn Phe Val Ser Ala Phe Leu Ala Ser Cys Lys Asp Gly Gly Thr  
 420 425 430  
 Leu Pro Asn Ala Gly Val Thr Ser Asn Thr Trp Gly Ala Gly Cys Ala  
 435 440 445  
 Tyr Val Gly Glu Thr Ile Ser Ala Leu Thr Asn Ser Ile Ala His Phe  
 450 455 460  
 Gly Thr Gln Glu Gln Gln Ile Gln Gln Ala Glu Asn Ile Ala Asp Thr  
 465 470 475 480  
 Leu Val Asn Phe Lys Ser Arg Tyr Ser Glu Leu Gly Asn Thr Tyr Asn  
 485 490 495  
 Ser Ile Thr Thr Ala Leu Ser Lys Val Pro Asn Ala Gln Ser Leu Gln  
 500 505 510  
 Asn Val Val Ser Lys Lys Asn Asn Pro Tyr Ser Pro Gln Gly Ile Glu  
 515 520 525  
 Thr Asn Tyr Tyr Leu Asn Gln Asn Ser Tyr Asn Gln Ile Gln Thr Ile  
 530 535 540  
 Asn Gln Glu Leu Gly Arg Asn Pro Phe Arg Lys Val Gly Ile Val Asn  
 545 550 555 560  
 Ser Gln Thr Asn Asn Gly Ala Met Asn Gly Ile Gly Ile Gln Val Gly  
 565 570 575  
 Tyr Lys Gln Phe Phe Gly Gln Lys Arg Lys Trp Gly Ala Arg Tyr Tyr

WO 00/00614

PCT/US99/14375

|                                                                 |     |     |
|-----------------------------------------------------------------|-----|-----|
| 580                                                             | 585 | 590 |
| Gly Phe Phe Asp Tyr Asn His Ala Phe Ile Lys Ser Ser Phe Phe Asn |     |     |
| 595                                                             | 600 | 605 |
| Ser Ala Ser Asp Val Trp Thr Tyr Gly Phe Gly Ala Asp Ala Leu Tyr |     |     |
| 610                                                             | 615 | 620 |
| Asn Phe Ile Asn Asp Lys Ala Thr Asn Phe Leu Gly Lys Asn Asn Lys |     |     |
| 625                                                             | 630 | 635 |
| Leu Ser Leu Gly Leu Phe Gly Gly Ile Ala Leu Ala Gly Thr Ser Trp |     |     |
| 645                                                             | 650 | 655 |
| Leu Asn Ser Glu Tyr Val Asn Leu Ala Thr Val Asn Asn Val Tyr Asn |     |     |
| 660                                                             | 665 | 670 |
| Ala Lys Met Asn Val Ala Asn Phe Gln Phe Leu Phe Asn Met Gly Val |     |     |
| 675                                                             | 680 | 685 |
| Arg Met Asn Leu Ala Arg Ser Lys Lys Lys Gly Ser Asp His Ala Ala |     |     |
| 690                                                             | 695 | 700 |
| Gln His Gly Ile Glu Leu Gly Leu Lys Ile Pro Thr Ile Asn Thr Asn |     |     |
| 705                                                             | 710 | 715 |
| Tyr Tyr Ser Phe Met Gly Ala Glu Leu Lys Tyr Arg Arg Leu Tyr Ser |     |     |
| 725                                                             | 730 | 735 |
| Val Tyr Leu Asn Tyr Val Phe Ala Tyr                             |     |     |
| 740                                                             | 745 |     |

## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2127 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| ATGAAAAAAAA CCCTTTTACT CTCTCTCTCT CTCTCTCTCT CGTTTTTGCT CCACGCTGAA | 60  |
| GACGACGGCT TTTACACAAG CGTGGGCTAT CAAATCGGTG AAGCCGCTCA AATGGTGAAA  | 120 |
| AACACCAAAG GCATTCAAGA GCTTTCAGAC AATTATGAAA AGCTGAACAA TCTTTTGAAT  | 180 |
| AATTACAGCA CCCTAAACAC CCTTATCAAA TTGTCCGCTG ATCCGAGCGC GATTAACGAC  | 240 |
| GCAAGGGATA ATCTAGGCTC AAGCTCTAGG AATTGCTTG ATGTCAAAC CAATCCCCC     | 300 |
| GCGTATCAAG CCGTGCTTTT AGCACTCAAT GCTGCAGTGG GGTGTGGCA AGTTACAAGC   | 360 |
| TACGCTTTTA CTGCTTGTGG TCCTGGCAGT AACGAGAATG CGAATGGAGG GATCCAAACT  | 420 |

WO 00/00614

PCT/US99/14375

|                                                                    |      |
|--------------------------------------------------------------------|------|
| TTTAATAATG TGCCAGGACA AGATACGACG ACCATCACTT GCAATTTCGTA TTATGAGCCA | 480  |
| GGACATGGTG GGCCTATATC CACTGCAAAT TATGCGAAAA TCAATCAAGC CTATCAAATC  | 540  |
| ATCCAAAAGG CTTTGACAGC CAATGGAGCT AATGGAGATG GGGTCCCCGT TTTAAGCAAC  | 600  |
| ACCACTACAA AACTTGATTT CACTATCAAT GGAGACAAAA GAACGGGGGG CAAACCAAAT  | 660  |
| ACACCTGAAA AGTTCCTATG GAGTGATGGG AAATATATTC ACACCCAATG GATTAACACA  | 720  |
| ATAGTAACAC CAACAGAAAC AAATATCAAC ACAGAAAATA ACGCTCAAGA GCTTTTAAAA  | 780  |
| CAAGCGAGCA TCATTATCAC TACCCTAAAT GAGGCATGCC CAAACTTCCA AAATGGTGGT  | 840  |
| AGAAGTTATT GGCAAGGGAT AAGCGGCAAT GGGACAATGT GCGGGATGTT TAAGAATGAA  | 900  |
| ATCAGCGCGA TCCAAGGCAT GATCGCTAAC GCTCAAGAAG CTGTCGCGCA AAGCAAAATC  | 960  |
| GTTAGTGAAA ACGCGCAAAA TCAAAACAAC TTGGATACTG GAAAACCATT CAACCCTTAC  | 1020 |
| ACGGACGCCA GCTTTGCGCA AAGCATGCTC AAAAACGCTC AAGCGCAAGC AGAGATTTTA  | 1080 |
| AACCAAGCCG AACAAGTAGT AAAAACTTT GAAAAATCC CTACAGCCTT TGTATCAGAC    | 1140 |
| TCTTTAGGGG TGTGTTATGA AGTGCAAGGG GGTGAGCGTA GGGGCACCAA TCCAGGTCAG  | 1200 |
| GTAACCTCTA AACTTGGGG AGCCGGTTGC GCGTATGTGA AACAAACCAT AACGAATTTA   | 1260 |
| GACAACAGCA TCGCTCACTT TGGCACTCAA GAGCAGCAGA TACAGCAAGC CGAAAACATC  | 1320 |
| GCTGACACTC TAGTGAATTT CAAATCTAGA TACAGCGAAT TAGGCAACAC CTATAACAGC  | 1380 |
| ATCACCACCG CGCTCTCCAA AGTCCCTAAC GCGCAAAGCT TGCAAAACGT GGTGAGCAAA  | 1440 |
| AAGAATAACC CCTATAGCCC TCAAGGCATA GAGACCAATT ACTACCTCAA TCAAAATTCT  | 1500 |
| TACAACCAAA TCCAACCAT CAACCAAGAA CTAGGGCGTA ACCCCTTTAG GAAAGTGGGC   | 1560 |
| ATCGTCAATT CTCAAACCAA CAATGGTGCC ATGAATGGGA TCGGTATTCA GGTGGGCTAT  | 1620 |
| AAGCAATTCT TTGGCCAAA AAGAAAATGG GGCCTAGGT ATTACGGCTT TTTTGACTAC    | 1680 |
| AACCATGCGT TCATTAAATC CAGCTTCTTC AACTCGGCTT CTGATGTGTG GACTTATGGT  | 1740 |
| TTTGAGCGG ACGCTCTTTA TAACTTCATC AACGATAAAG CCACCAATTT CTTAGGCAAA   | 1800 |
| AACAACAAGC TTTCCGTGGG GCTTTTGGGA GGGATTGCGT TAGCGGGCAC TTCATGGCTT  | 1860 |
| AATTCTGAGT ATGTGAATTT AGCCACCGTG AATAACGTCT ATAACGCTAA AATGAATGTG  | 1920 |
| GCGAATTTCC AATTCTTATT CAATATGGGA GTGAGGATGA ATTTAGCCAG ATCCAAGAAA  | 1980 |
| AAAGGCAGCG ATCATGCGGC TCAGCATGGG ATTGAACTAG GGCTTAAAT CCCCACCATC   | 2040 |
| AACACGAACT ATTATTCTTT CATGGGGGCT GAACTCAAAT ACAGAAGGCT TTATAGCGTG  | 2100 |
| TATTTGAATT ATGTGTTTCG TACTAA                                       | 2127 |

WO 00/00614

PCT/US99/14375

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2226 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

|                                                                    |      |
|--------------------------------------------------------------------|------|
| ATGAAAAAAC ACATCCTTTC ATTAGCTTTA GGCTCGCTTT TAGTTTCCAC TTTGAGCGCT  | 60   |
| GAAGACGACG GCTTTTACAC AAGCGTGGGC TATCAAATCG GTGAAGCCGC TCAAATGGTA  | 120  |
| ACAAACACCA AAGGCATTCA AGATCTTTCA GATCGTTATG AAAGTTTGAA CAACCTTTTG  | 180  |
| ACCCGATACA GCACCCTAAA CACCCTGATC AAATTGTCCG CTGATCCGAG CGCGATTAAT  | 240  |
| GCGGCGCGTG AAAATCTGGG CGCGAGCGCG AAGAATTTGA TCGGCGATAA AGCCAATTCC  | 300  |
| CCAGCCTATC AAGCGGTGCT TTTAGCGATC AACGCGGCGG TAGGGTTTTG GAATGTCTTA  | 360  |
| GGCTATGCTA CGCAATGCGG GGGTAACGCC AATGGTCAAA AAAGCACCTC TTCAACGACC  | 420  |
| ATCTTCAACA ACGAGCCAGG GTATCGATCC ACTTCCATCA CTGCTCTTT GAACGGGTAT   | 480  |
| ACGCCGTGGAT ACTATGGCCC TATGAGTATT GAGAATTTCA AAAAGCTTAA CGAAGCCTAT | 540  |
| CAGATCCTCC AAACGGCGTT AAAACAAGGC TTACCCGCGC TCAAAGAAAA CAACAAGAAG  | 600  |
| GTCAATGTTA CCTACACTTA CACATGCTCA GGGGGAGGGA ATAATAACTG CTCGTCAGAA  | 660  |
| GCCACAGGTG TAAGCAATCA AAATGGCGGA ACTAAAACCA CCACCCAAAC CATAGACGGC  | 720  |
| AAAAGCGTAA CCACCACGAT CAGTTCAAAA GTCGTTGATA GCACAGCGAG TGGTAACACA  | 780  |
| TCACGTGTCT CCTACACCGA AATCACCAC AAATTAGAAG GTGTGCCTGA TAGCGCTCAA   | 840  |
| GCGCTCTTAG CGCAAGCGAG CACGCTCATT AGCACCATCA ACACGGCATG CCCGTTTTTT  | 900  |
| AGTGTAAC TAACAAAGTGG TGGTCCACAG ATGGAACCGA CTAAAGGGAA GTTGTGCGGT   | 960  |
| TTTACAGAAG AAATCAGCGC GATCCAAAAG ATGATCACAG ACGCGCAAGA GCTGGTCAAT  | 1020 |
| CAAACGAGCG TCATTAATAG CCATGAACAA TCAACCCTAG TGGGCGGTAA TAATGGCAAG  | 1080 |
| CCTTTCAACC CTTTCACGGA CGCTCAATTC GCTCAAGGCA TGCTCGCTAA CGCTAGCGCG  | 1140 |
| CAAGCTAAAA TGCTCAATTT AGCCCATCAA GTGGGGCAAA CCATTAACCC TAACAATCTT  | 1200 |
| ACTGGGAATT TTAAAAATTT TGTTACAGGC TTTT TAGCCA CATGCAACAA CCCCTCAACA | 1260 |
| GCTGGCACTG GTGGCACACA AGGTTAGCT CCAGGCACGG TTACCACTCA AACTTTTCGT   | 1320 |
| TCCGGTTGCG CGTATGTGGA ACAAACCATA ACGAATTTAG AAAACAGCAT CGCGCACTTT  | 1380 |

WO 00/00614

PCT/US99/14375

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GGCACTCAAG AGCAGCAAAT ACAACGAGCC GAAAATATCG CTGACACTCT AGTGAATTTT 1440
AAATCTAGAT ACAGCGAATT GGGGAATACT TACAACAGCA TCACCACTGC GCTCTCCAAA 1500
GTCCCTAACG CGCAAAGCTT GCAAAACGTG GTGAGCAAAA AGAATAACCC CTATAGCCCC 1560
CAAGGCATAG AAACCAATTA CTACTTGAAT CAAAATTCTT ACAACCAAAT CCAAACCATC 1620
AACCAAGAAT TAGGGCGTAA CCCTTTTAGG AAAGTGGGCA TCGTCGGCTC TCAAACCAAC 1680
AACGGCGCCA TGAATGGGAT CGGTATTCAG GTGGGCTACG AGCAATTCTT TGGCCAAAAA 1740
AGAAAATGGG GCGCTAGGTA TTACGGCTTT TTTGATTACA ACCATGCGTT TATTAAATCC 1800
AGCTTCTTCA ACTCGGCTTC TGATGTGTGG ACTTATGGTT TTGGAGCGGA CGCTCTCTAT 1860
AACTTCATCA ACGATAAAGC CACTAACTTT TTAGGCAAAA ACAACAAGCT TTCTGTGGGG 1920
CTTTTTGGCG GGATTGCGTT AGCGGGCACT TCATGGCTTA ATTCTGAGTA TGTGAATTTA 1980
GCCACCGTGA ATAATGTCTA TAACGCTAAA ATGAACGTGG CGAACTTCCA ATTCTTATTC 2040
AACATGGGAG TGAGGATGAA TTTGGCCAGG CCCAAGAAAA ACGACAGCGA TCATGCGGCT 2100
CAGCATGGGA TTGAGTTAGG GCTTAAAATC CCCACCATCA ACACGAACTA CTATTCCTTT 2160
ATGGGGGCTG AACTCAAATA CAGAAGGCTT TATAGCGTGT ATTTGAATTA TGTGTTGCT 2220
TACTAG 2226

```

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2238 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

ATGAAAAAAC ACATCCTTTC ATTAGCTTTA GGCTCGCTTT TAGTTTCCAC TTTGAGCGCT 60
GAAGACGACG GCTTTTACAC AAGCGTAGGC TATCAGATCG GTGAAGCCGC TCAAATGGTA 120
ACAAACACCA AAGGCATCCA ACAGCTTTCA GACAATTATG AAAATTTGAA CAACCTTTTA 180
ACGAGATACA GCACCCTAAA CACCCTTATC AAATTGTCCG CTGATCCGAG CGCAATTAAT 240
GCGGTGCGGG AAAATCTGGG CGCGAGCACG AAGAATTTGA TCGGCGATAA AGCCAACTCC 300
CCGGCGTATC AAGCCGTGTT TTTAGCGATC AACGCGGCGG TAGGGTTGTG GAATACCATC 360
GGCTATGCGG TCATGTGCGG GAACGGGAAC GGCACAGAGA GTGGGCCTGG CAGCGTGATC 420

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WO 00/00614

PCT/US99/14375

|                                                                    |      |
|--------------------------------------------------------------------|------|
| TTTAATGACC AACCAGGACA GGATTCCACG CAAATTACTT GCAACCGCTT TGAATCAACT  | 480  |
| GGGCCTGGTA AAAGCATGTC TATTGATGAA TTCAAAAAC TCAATGAAGC CTATCAAATC   | 540  |
| ATCCAGCAAG CTTTAAAAA TCAAAGTGGG TTTCCTGAAT TAGGCGGGAA CGGCACAAAA   | 600  |
| GTGAGTGTTA ATTACAATTA CGAATGCAGA CAACTGCTG ATATCAACGG CGGTGTGTAT   | 660  |
| CAGTTCTGCA AGGCTAAAAA TGGTAGTAGT AGCAGTAGTA ATGGCGGTAA TGGCAGTAGC  | 720  |
| ACGCAAACAA CCGCGACAAC CACGCAAGAC GGC GTAACGA TCACCACTAC CTATAATAAT | 780  |
| AACAAAGCCA CCGTCAAATT TGACATCACC AATAACGCTG AACAGCTGTT AAATCAAGCG  | 840  |
| GCAAACATCA TGCAAGTCCT TAATACGCAA TGCCCTTTAG TGC GTTCCAC GAATAACGAA | 900  |
| AACACTCCAG GGGGTGGTCA ACCATGGGGT TTAAGCACAT CCGGGAATGC GTGCAGCATC  | 960  |
| TTCCAACAAG AATTTAGCCA GGTTACTAGC ATGATCAAAA ACGCCAAGA AATAATCGCG   | 1020 |
| CAAAGCAAAA TCGTTAGTGA AAACGCGCAA AATCAAAACA ACTTGGATAC TGGAAAACCA  | 1080 |
| TTCAACCCTT ACACGGACGC CAGCTTTGCG CAAAGCATGC TCAAAAACGC TCAAGCGCAA  | 1140 |
| GCAGAGATGT TCAATTTGAG CGAACAAGTG AAAAAGAACT TGGAAGTCAT GAAAAACAAC  | 1200 |
| AATAATGTTA ACGAGAAATT AGCAGGATTT GGGAAAGAAG AAGTAATGAC CAATTTTGTT  | 1260 |
| AGCGCCTTTT TGGCAAGCTG CAAAGATGGT GGCACATTGC CTAATGCAGG GGTTACTTCT  | 1320 |
| AACACTTGGG GGGCGGGTTG CGCGTATGTG GGAGAGACGA TAAGCGCCCT AACCAACAGC  | 1380 |
| ATCGCTCACT TTGGCACTCA AGAGCAGCAG ATACAGCAAG CCGAAAACAT CGCTGACACT  | 1440 |
| CTAGTGAATT TCAAATCTAG ATACAGCGAA TTAGGCAACA CCTATAACAG CATCACCACC  | 1500 |
| GCGCTCTCCA AAGTCCCTAA CGCGCAAAGC TTGCAAAACG TGGTGAGCAA AAAGAATAAC  | 1560 |
| CCCTATAGCC CTCAAGGCAT AGAGACCAAT TACTACCTCA ATCAAAATTC TTACAACCAA  | 1620 |
| ATCCAAACCA TCAACCAAGA ACTAGGGCGT AACCCCTTTA GGAAAGTGGG CATCGTCAAT  | 1680 |
| TCTCAAACCA ACAATGGTGC CATGAATGGG ATCGGCATTC AGGTGGGCTA TAAGCAATTC  | 1740 |
| TTTGGCCAAA AAAGAAAATG GGGCGCTAGG TATTACGGCT TTTTGTGATTA CAACCATGCG | 1800 |
| TTCATCAAAT CCAGCTTTTT CAACTCGGCT TCTGACGTGT GGACTTATGG TTTTGAGCG   | 1860 |
| GACGCGCTTT ATAACCTCAT CAACGATAAA GCCACCAATT TCTTAGGCAA AAACAACAAG  | 1920 |
| CTTTCTTTGG GGCTTTTTGG CGGGATTGCG TTAGCGGGCA CTTCATGGCT CAATTCTGAG  | 1980 |
| TACGTGAATT TAGCCACCGT GAATAACGTC TATAACGCTA AAATGAATGT GGCGAATTC   | 2040 |
| CAATTCTTAT TCAATATGGG AGTGAGGATG AATTTAGCCA GATCCAAGAA AAAAGGCAGC  | 2100 |
| GATCATGCAG CTCAGCATGG GATTGAGTTA GGGCTTAAAA TCCCCACCAT CAACACGAAC  | 2160 |
| TATTATTCCT TTATGGGGGC TGAAC TCAAA TACAGAAGGC TCTATAGCGT GTATTTGAAC | 2220 |



TATGTGTTTCG CTTACTAA

2238

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Asp | Asp | Gly | Phe | Tyr | Thr | Ser | Val | Gly | Tyr | Gln | Ile | Gly | Glu | Ala |
| 1   |     |     | 5   |     |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Ala | Gln | Met | Val |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     | 20  |     |     |     |     |     |     |     |     |     |     |     |     |     |

## (2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 39 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GARGAYGAYG GNTTYTAYAC NWSNGTNGGN TAYCARATHG GNGARGCNGC NCARATGGTN 60

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 42 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGCCATATGA AAAAACACAT CCTTTCATTA GCTTTAGGCT CG 42

## (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:

WO 00/00614

PCT/US99/14375

- (A) LENGTH: 42 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGCAAGCTTG GGAGTTTCAC AAAAAGCTTA GTAAGCGAAC AC

42

09/720560

528 Rec'd PCT/PTO 21 DEC 2000

## SEQUENCE LISTING

<110> American Cyanamid Company  
Fulginiti, James P.  
Fiske, Michael J.  
Dilts, Deborah A.

&lt;120&gt; NOVEL ANTIGENS OF HELICOBACTER PYLORI

<130> 0646/89939

<140> PCT/US99/14375

<141> 1999-06-25

<150> 60/090,851

<151> 1998-06-26

<160> 42

<170> FastSEQ for Windows Version 3.0

<210> 1

<211> 708

<212> PRT

<213> Helicobacter pylori

<400> 1

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|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Lys | Lys | Thr | Leu | Leu | Leu | Ser | Leu | Ser | Leu | Ser | Leu | Ser | Phe | Leu |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |
| Leu | His | Ala | Glu | Asp | Asp | Gly | Phe | Tyr | Thr | Ser | Val | Gly | Tyr | Gln | Ile |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     |     | 30  |     |
| Gly | Glu | Ala | Ala | Gln | Met | Val | Lys | Asn | Thr | Lys | Gly | Ile | Gln | Glu | Leu |
|     |     |     | 35  |     |     |     | 40  |     |     |     |     | 45  |     |     |     |
| Ser | Asp | Asn | Tyr | Glu | Lys | Leu | Asn | Asn | Leu | Leu | Asn | Asn | Tyr | Ser | Thr |
|     |     |     | 50  |     |     | 55  |     |     |     |     | 60  |     |     |     |     |
| Leu | Asn | Thr | Leu | Ile | Lys | Leu | Ser | Ala | Asp | Pro | Ser | Ala | Ile | Asn | Asp |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     | 80  |     |
| Ala | Arg | Asp | Asn | Leu | Gly | Ser | Ser | Ser | Arg | Asn | Leu | Leu | Asp | Val | Lys |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |
| Thr | Asn | Ser | Pro | Ala | Tyr | Gln | Ala | Val | Leu | Leu | Ala | Leu | Asn | Ala | Ala |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
| Val | Gly | Leu | Trp | Gln | Val | Thr | Ser | Tyr | Ala | Phe | Thr | Ala | Cys | Gly | Pro |
|     |     |     | 115 |     |     |     | 120 |     |     |     |     | 125 |     |     |     |
| Gly | Ser | Asn | Glu | Asn | Ala | Asn | Gly | Gly | Ile | Gln | Thr | Phe | Asn | Asn | Val |
|     |     |     | 130 |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
| Pro | Gly | Gln | Asp | Thr | Thr | Ile | Thr | Cys | Asn | Ser | Tyr | Tyr | Glu | Pro |     |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     | 160 |     |
| Gly | His | Gly | Gly | Pro | Ile | Ser | Thr | Ala | Asn | Tyr | Ala | Lys | Ile | Asn | Gln |
|     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |
| Ala | Tyr | Gln | Ile | Ile | Gln | Lys | Ala | Leu | Thr | Ala | Asn | Gly | Ala | Asn | Gly |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |
| Asp | Gly | Val | Pro | Val | Leu | Ser | Asn | Thr | Thr | Thr | Lys | Leu | Asp | Phe | Thr |
|     |     |     | 195 |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
| Ile | Asn | Gly | Asp | Lys | Arg | Thr | Gly | Gly | Lys | Pro | Asn | Thr | Pro | Glu | Lys |
|     |     |     | 210 |     |     | 215 |     |     |     |     | 220 |     |     |     |     |

Phe Pro Trp Ser Asp Gly Lys Tyr Ile His Thr Gln Trp Ile Asn Thr  
 225 230 235 240  
 Ile Val Thr Pro Thr Glu Thr Asn Ile Asn Thr Glu Asn Asn Ala Gln  
 245 250 255  
 Glu Leu Leu Lys Gln Ala Ser Ile Ile Thr Thr Leu Asn Glu Ala  
 260 265 270  
 Cys Pro Asn Phe Gln Asn Gly Gly Arg Ser Tyr Trp Gln Gly Ile Ser  
 275 280 285  
 Gly Asn Gly Thr Met Cys Gly Met Phe Lys Asn Glu Ile Ser Ala Ile  
 290 295 300  
 Gln Gly Met Ile Ala Asn Ala Gln Glu Ala Val Ala Gln Ser Lys Ile  
 305 310 315 320  
 Val Ser Glu Asn Ala Gln Asn Gln Asn Asn Leu Asp Thr Gly Lys Pro  
 325 330 335  
 Phe Asn Pro Tyr Thr Asp Ala Ser Phe Ala Gln Ser Met Leu Lys Asn  
 340 345 350  
 Ala Gln Ala Gln Ala Glu Ile Leu Asn Gln Ala Glu Gln Val Val Lys  
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 Asn Phe Glu Lys Ile Pro Thr Ala Phe Val Ser Asp Ser Leu Gly Val  
 370 375 380  
 Cys Tyr Glu Val Gln Gly Gly Glu Arg Arg Gly Thr Asn Pro Gly Gln  
 385 390 395 400  
 Val Thr Ser Asn Thr Trp Gly Ala Gly Cys Ala Tyr Val Lys Gln Thr  
 405 410 415  
 Ile Thr Asn Leu Asp Asn Ser Ile Ala His Phe Gly Thr Gln Glu Gln  
 420 425 430  
 Gln Ile Gln Gln Ala Glu Asn Ile Ala Asp Thr Leu Val Asn Phe Lys  
 435 440 445  
 Ser Arg Tyr Ser Glu Leu Gly Asn Thr Tyr Asn Ser Ile Thr Thr Ala  
 450 455 460  
 Leu Ser Lys Val Pro Asn Ala Gln Ser Leu Gln Asn Val Val Ser Lys  
 465 470 475 480  
 Lys Asn Asn Pro Tyr Ser Pro Gln Gly Ile Glu Thr Asn Tyr Tyr Leu  
 485 490 495  
 Asn Gln Asn Ser Tyr Asn Gln Ile Gln Thr Ile Asn Gln Glu Leu Gly  
 500 505 510  
 Arg Asn Pro Phe Arg Lys Val Gly Ile Val Asn Ser Gln Thr Asn Asn  
 515 520 525  
 Gly Ala Met Asn Gly Ile Gly Ile Gln Val Gly Tyr Lys Gln Phe Phe  
 530 535 540  
 Gly Gln Lys Arg Lys Trp Gly Ala Arg Tyr Tyr Gly Phe Phe Asp Tyr  
 545 550 555 560  
 Asn His Ala Phe Ile Lys Ser Ser Phe Phe Asn Ser Ala Ser Asp Val  
 565 570 575  
 Trp Thr Tyr Gly Phe Gly Ala Asp Ala Leu Tyr Asn Phe Ile Asn Asp  
 580 585 590  
 Lys Ala Thr Asn Phe Leu Gly Lys Asn Asn Lys Leu Ser Val Gly Leu  
 595 600 605  
 Phe Gly Gly Ile Ala Leu Ala Gly Thr Ser Trp Leu Asn Ser Glu Tyr  
 610 615 620  
 Val Asn Leu Ala Thr Val Asn Asn Val Tyr Asn Ala Lys Met Asn Val  
 625 630 635 640  
 Ala Asn Phe Gln Phe Leu Phe Asn Met Gly Val Arg Met Asn Leu Ala  
 645 650 655  
 Arg Ser Lys Lys Lys Gly Ser Asp His Ala Ala Gln His Gly Ile Glu  
 660 665 670  
 Leu Gly Leu Lys Ile Pro Thr Ile Asn Thr Asn Tyr Tyr Ser Phe Met



|                             |                         |                     |     |     |
|-----------------------------|-------------------------|---------------------|-----|-----|
| 355                         |                         | 360                 |     | 365 |
| Gln Phe Ala Gln Gly Met     | Leu Ala Asn Ala Ser     | Ala Gln Ala Lys Met |     |     |
| 370                         | 375                     | 380                 |     |     |
| Leu Asn Leu Ala His Gln     | Val Gly Gln Thr Ile     | Asn Pro Asn Asn Leu |     |     |
| 385                         | 390                     | 395                 | 400 |     |
| Thr Gly Asn Phe Lys Asn Phe | Val Thr Gly Phe Leu Ala | Thr Cys Asn         |     |     |
|                             | 405                     | 410                 | 415 |     |
| Asn Pro Ser Thr Ala Gly     | Thr Gly Gly Thr Gln Gly | Ser Ala Pro Gly     |     |     |
|                             | 420                     | 425                 | 430 |     |
| Thr Val Thr Thr Gln Thr     | Phe Ala Ser Gly Cys Ala | Tyr Val Glu Gln     |     |     |
|                             | 435                     | 440                 | 445 |     |
| Thr Ile Thr Asn Leu Glu     | Asn Ser Ile Ala His     | Phe Gly Thr Gln Glu |     |     |
|                             | 450                     | 455                 | 460 |     |
| Gln Gln Ile Gln Arg Ala     | Glu Asn Ile Ala Asp     | Thr Leu Val Asn Phe |     |     |
| 465                         | 470                     | 475                 | 480 |     |
| Lys Ser Arg Tyr Ser Glu     | Leu Gly Asn Thr Tyr     | Asn Ser Ile Thr Thr |     |     |
|                             | 485                     | 490                 | 495 |     |
| Ala Leu Ser Lys Val Pro     | Asn Ala Gln Ser Leu     | Gln Asn Val Val Ser |     |     |
|                             | 500                     | 505                 | 510 |     |
| Lys Lys Asn Asn Pro Tyr     | Ser Pro Gln Gly Ile     | Glu Thr Asn Tyr Tyr |     |     |
|                             | 515                     | 520                 | 525 |     |
| Leu Asn Gln Asn Ser Tyr     | Asn Gln Ile Gln Thr     | Ile Asn Gln Glu Leu |     |     |
|                             | 530                     | 535                 | 540 |     |
| Gly Arg Asn Pro Phe Arg     | Lys Val Gly Ile Val     | Gly Ser Gln Thr Asn |     |     |
| 545                         | 550                     | 555                 | 560 |     |
| Asn Gly Ala Met Asn Gly     | Ile Gly Ile Gln Val     | Gly Tyr Glu Gln Phe |     |     |
|                             | 565                     | 570                 | 575 |     |
| Phe Gly Gln Lys Arg Lys     | Trp Gly Ala Arg Tyr     | Tyr Gly Phe Phe Asp |     |     |
|                             | 580                     | 585                 | 590 |     |
| Tyr Asn His Ala Phe Ile     | Lys Ser Ser Phe Phe     | Asn Ser Ala Ser Asp |     |     |
|                             | 595                     | 600                 | 605 |     |
| Val Trp Thr Tyr Gly Phe     | Gly Ala Asp Ala Leu     | Tyr Asn Phe Ile Asn |     |     |
|                             | 610                     | 615                 | 620 |     |
| Asp Lys Ala Thr Asn Phe     | Leu Gly Lys Asn Asn     | Lys Leu Ser Val Gly |     |     |
| 625                         | 630                     | 635                 | 640 |     |
| Leu Phe Gly Gly Ile Ala     | Leu Ala Gly Thr Ser     | Trp Leu Asn Ser Glu |     |     |
|                             | 645                     | 650                 | 655 |     |
| Tyr Val Asn Leu Ala Thr     | Val Asn Asn Val Tyr     | Asn Ala Lys Met Asn |     |     |
|                             | 660                     | 665                 | 670 |     |
| Val Ala Asn Phe Gln Phe     | Leu Phe Asn Met Gly     | Val Arg Met Asn Leu |     |     |
|                             | 675                     | 680                 | 685 |     |
| Ala Arg Pro Lys Lys Asn     | Asp Ser Asp His Ala     | Ala Gln His Gly Ile |     |     |
|                             | 690                     | 695                 | 700 |     |
| Glu Leu Gly Leu Lys Ile     | Pro Thr Ile Asn Thr     | Asn Tyr Tyr Ser Phe |     |     |
| 705                         | 710                     | 715                 | 720 |     |
| Met Gly Ala Glu Leu Lys     | Tyr Arg Arg Leu Tyr     | Ser Val Tyr Leu Asn |     |     |
|                             | 725                     | 730                 | 735 |     |
| Tyr Val Phe Ala Tyr         |                         |                     |     |     |
| 740                         |                         |                     |     |     |

&lt;210&gt; 3

&lt;211&gt; 745

&lt;212&gt; PRT

&lt;213&gt; Helicobacter pylori

&lt;400&gt; 3

Met Lys Lys His Ile Leu Ser Leu Ala Leu Gly Ser Leu Leu Val Ser

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |  |
| Thr | Leu | Ser | Ala | Glu | Asp | Asp | Gly | Phe | Tyr | Thr | Ser | Val | Gly | Tyr | Gln |  |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |  |
| Ile | Gly | Glu | Ala | Ala | Gln | Met | Val | Thr | Asn | Thr | Lys | Gly | Ile | Gln | Gln |  |
|     |     |     | 35  |     |     |     | 40  |     |     |     |     | 45  |     |     |     |  |
| Leu | Ser | Asp | Asn | Tyr | Glu | Asn | Leu | Asn | Asn | Leu | Leu | Thr | Arg | Tyr | Ser |  |
|     |     |     | 50  |     |     | 55  |     |     |     |     | 60  |     |     |     |     |  |
| Thr | Leu | Asn | Thr | Leu | Ile | Lys | Leu | Ser | Ala | Asp | Pro | Ser | Ala | Ile | Asn |  |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |  |
| Ala | Val | Arg | Glu | Asn | Leu | Gly | Ala | Ser | Thr | Lys | Asn | Leu | Ile | Gly | Asp |  |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |  |
| Lys | Ala | Asn | Ser | Pro | Ala | Tyr | Gln | Ala | Val | Phe | Leu | Ala | Ile | Asn | Ala |  |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |  |
| Ala | Val | Gly | Leu | Trp | Asn | Thr | Ile | Gly | Tyr | Ala | Val | Met | Cys | Gly | Asn |  |
|     |     |     | 115 |     |     |     |     | 120 |     |     |     | 125 |     |     |     |  |
| Gly | Asn | Gly | Thr | Glu | Ser | Gly | Pro | Gly | Ser | Val | Ile | Phe | Asn | Asp | Gln |  |
|     |     |     | 130 |     |     | 135 |     |     |     |     | 140 |     |     |     |     |  |
| Pro | Gly | Gln | Asp | Ser | Thr | Gln | Ile | Thr | Cys | Asn | Arg | Phe | Glu | Ser | Thr |  |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |  |
| Gly | Pro | Gly | Lys | Ser | Met | Ser | Ile | Asp | Glu | Phe | Lys | Lys | Leu | Asn | Glu |  |
|     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |  |
| Ala | Tyr | Gln | Ile | Gln | Gln | Ala | Leu | Lys | Asn | Gln | Ser | Gly | Phe | Pro |     |  |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |  |
| Glu | Leu | Gly | Gly | Asn | Gly | Thr | Lys | Val | Ser | Val | Asn | Tyr | Asn | Tyr | Glu |  |
|     |     |     | 195 |     |     |     | 200 |     |     |     |     | 205 |     |     |     |  |
| Cys | Arg | Gln | Thr | Ala | Asp | Ile | Asn | Gly | Gly | Val | Tyr | Gln | Phe | Cys | Lys |  |
|     |     |     | 210 |     |     | 215 |     |     |     |     | 220 |     |     |     |     |  |
| Ala | Lys | Asn | Gly | Ser | Ser | Ser | Ser | Ser | Asn | Gly | Gly | Asn | Gly | Ser | Ser |  |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |  |
| Thr | Gln | Thr | Thr | Ala | Thr | Thr | Thr | Gln | Asp | Gly | Val | Thr | Ile | Thr | Thr |  |
|     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |  |
| Thr | Tyr | Asn | Asn | Asn | Lys | Ala | Thr | Val | Lys | Phe | Asp | Ile | Thr | Asn | Asn |  |
|     |     |     |     | 260 |     |     |     | 265 |     |     |     |     | 270 |     |     |  |
| Ala | Glu | Gln | Leu | Leu | Asn | Gln | Ala | Ala | Asn | Ile | Met | Gln | Val | Leu | Asn |  |
|     |     |     |     | 275 |     |     | 280 |     |     |     |     |     | 285 |     |     |  |
| Thr | Gln | Cys | Pro | Leu | Val | Arg | Ser | Thr | Asn | Asn | Glu | Asn | Thr | Pro | Gly |  |
|     |     |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |  |
| Gly | Gly | Gln | Pro | Trp | Gly | Leu | Ser | Thr | Ser | Gly | Asn | Ala | Cys | Ser | Ile |  |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |  |
| Phe | Gln | Gln | Glu | Phe | Ser | Gln | Val | Thr | Ser | Met | Ile | Lys | Asn | Ala | Gln |  |
|     |     |     |     | 325 |     |     |     |     |     | 330 |     |     |     | 335 |     |  |
| Glu | Ile | Ile | Ala | Gln | Ser | Lys | Ile | Val | Ser | Glu | Asn | Ala | Gln | Asn | Gln |  |
|     |     |     | 340 |     |     |     |     | 345 |     |     |     |     |     |     |     |  |





|             |             |             |            |             |            |      |
|-------------|-------------|-------------|------------|-------------|------------|------|
| agaagttatt  | ggcaagggat  | aagcggcaat  | gggacaatgt | gcgggatggt  | taagaatgaa | 900  |
| atcagcgcga  | tccaagggcat | gatcgctaac  | gctcaagaag | ctgtcgcgca  | aagcaaaatc | 960  |
| gttagtgaaa  | acgcgcacaaa | tcaaaacaac  | ttggatactg | gaaaaccatt  | caacccttac | 1020 |
| acggacgcc   | gctttgcgca  | aagcatgctc  | aaaaacgctc | aagcgcaagc  | agagatttta | 1080 |
| aaccaagcgg  | aacaagtagt  | aaaaaacttt  | gaaaaaatcc | ctacagcctt  | tgtatcagac | 1140 |
| tcttttagggg | tgtgttatga  | agtgaagg    | ggtgagcgta | ggggcaccaa  | tccaggtcag | 1200 |
| gtaactttcta | acacttgggg  | agccggttgc  | gcgtatgtga | aacaaaccat  | aacgaattta | 1260 |
| gacaacagca  | tcgctcactt  | tggcactcaa  | gagcagcaga | tacagcaagc  | cgaaaacatc | 1320 |
| gctgacactc  | tagtgaattt  | caaactctaga | tacagcgaat | taggcaacac  | ctataacagc | 1380 |
| atcaccaccg  | cgctctccaa  | agtcctaac   | gcgcaaagct | tgcaaaacgt  | ggtgagcaaa | 1440 |
| aagaataacc  | cctatagccc  | tcaaggcata  | gagaccaatt | actacctcaa  | tcaaaattct | 1500 |
| tacaaccaaa  | tccaaaccat  | caaccaagaa  | ctagggcgta | accccttttag | gaaagtgggc | 1560 |
| atcgtcaatt  | ctcaaaccac  | caatgggtgc  | atgaatggga | tcggtattca  | ggtgggctat | 1620 |
| aagcaatttct | ttggccaaaa  | aagaaaatgg  | ggcgataggt | attacggctt  | ttttgactac | 1680 |
| aacctgctg   | tcattaaatc  | cagcttcttc  | aactcggtt  | ctgatgtgtg  | gacttatggt | 1740 |
| tttgagcgg   | acgctcttta  | taacttcac   | aacgataaag | ccaccaattt  | cttaggcaaa | 1800 |
| aacaacaagc  | tttcggtggg  | gctttttgga  | gggattgctg | tagcgggcac  | ttcatggctt | 1860 |
| aattctgagt  | atgtgaattt  | agccaccgtg  | aataacgtct | ataacgctaa  | aatgaatgtg | 1920 |
| gcgaattttcc | aattcttatt  | caatatggga  | gtgaggatga | atttagccag  | atccaagaaa | 1980 |
| aaaggcagcg  | atcatgcggc  | tcagcatggg  | attgaactag | ggcttaaaat  | ccccaccatc | 2040 |
| aacacgaact  | attattcttt  | catgggggct  | gaactcaaat | acagaaggct  | ttatagcgtg | 2100 |
| tatttgaatt  | atgtgttcgc  | ttacttaa    |            |             |            | 2127 |

<210> 5  
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 <213> Helicobacter pylori

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| gaagacgacg  | gcttttacac  | aagcgtgggc  | tatcaaatcg  | gtgaagccgc  | tcaaatggta  | 120  |
| acaaacacca  | aaggcattca  | agatctttca  | gatcggttatg | aaagtttgaa  | caaccttttg  | 180  |
| acccgataca  | gcaccctaaa  | caccctgatc  | aaattgtccg  | ctgatccgag  | cgcgattaat  | 240  |
| gcggcgcggtg | aaaatctggg  | cgcgagcgcg  | aagaatttga  | tcggcgataa  | agccaattcc  | 300  |
| ccagcctatc  | aagcgggtgct | tttagcgatc  | aacgcggcgg  | taggggttttg | gaatgtctta  | 360  |
| ggctatgcta  | cgcaatgcgg  | gggtaacgcc  | aatgggtcaaa | aaagcacctc  | ttcaacgacc  | 420  |
| atcttcaaca  | acgagccagg  | gtatcgatcc  | acttccatca  | cttgcctctt  | gaacgggtat  | 480  |
| acgcctggat  | actatggccc  | tatgagtatt  | gagaatttca  | aaaagcttaa  | cgaagcctat  | 540  |
| cagatcctcc  | aaacggcggt  | aaaacaaggc  | ttaccgcgcg  | tcaaagaaaa  | caacaagaag  | 600  |
| gtcaatgtta  | cctacactta  | cacatgctca  | gggggagggg  | ataataactg  | ctcgtcagaa  | 660  |
| gccacagggtg | taagcaatca  | aaatggcgga  | actaaaacca  | ccacccaaac  | catagacggc  | 720  |
| aaaagcgtaa  | ccaccacgat  | cagttcaaaa  | gtcgttgata  | gcacagcgag  | tggtaacaca  | 780  |
| tcacgtgtct  | cctacaccga  | aatcaccaac  | aaattagaag  | gtgtgcctga  | tagcgtctcaa | 840  |
| gcgctcttag  | cgcaagcgag  | cacgctcatt  | agcaccatca  | acacggcatg  | cccgtttttt  | 900  |
| agtgtaacta  | atcaaagtgg  | tgggtccacag | atggaaccga  | ctaaagggaa  | ggtgtgcggg  | 960  |
| tttacagaag  | aaatcagcgc  | gatccaaaag  | atgatcacag  | acgcgcaaga  | gctggtcaat  | 1020 |
| caaacgagcg  | tcattaatag  | ccatgaacaa  | tcaaccctag  | tgggcggtaa  | taatggcaag  | 1080 |
| cctttcaacc  | ctttcacgga  | cgctcaattc  | gctcaaggca  | tgtcgcgtaa  | cgctagcgcg  | 1140 |
| caagctaaaa  | tgtcctaatt  | agcccatcaa  | gtggggcaaa  | ccattaaccc  | taacaatctt  | 1200 |
| actgggaatt  | ttaaaaattt  | tgttacaggc  | tttttagcca  | catgcaacaa  | cccctcaaca  | 1260 |
| gctggcactg  | gtggcacaca  | aggttcagct  | ccaggcacgg  | ttaccactca  | aactttcgct  | 1320 |
| tccgggttgcg | cgtatgtgga  | acaaaccata  | acgaatttag  | aaaacagcat  | cgcgactttt  | 1380 |
| ggcactcaag  | agcagcaaat  | acaacgagcc  | gaaaatatcg  | ctgacactct  | agtgaatttc  | 1440 |
| aaatctagat  | acagcgaatt  | ggggaatact  | tacaacagca  | tcaccactgc  | gctctccaaa  | 1500 |
| gtccctaacg  | cgcaaagctt  | gcaaaacgtg  | gtgagcaaaa  | agaataaccc  | ctatagcccg  | 1560 |
| caaggcatag  | aaaccaatta  | ctacttgaat  | caaaattctt  | acaaccaaat  | ccaaaccatc  | 1620 |
| aaccaagaat  | tagggcgtaa  | cccttttagg  | aaagtgggca  | tcgtcggctc  | tcaaaccaac  | 1680 |



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<212> PRT
<213> Helicobacter pylori

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Ala Gln Met Val
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<212> DNA
<213> "Artificial sequence"

<400> 9
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                                     42

<210> 10
<211> 20
<212> DNA
<213> "Artificial sequence"

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                                     20

<210> 11
<211> 33
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<400> 11
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<210> 12
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<212> DNA
<213> "Artificial sequence"

<400> 12
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<210> 13
<211> 21
<212> DNA
<213> "Artificial sequence"

<400> 13
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<212> DNA
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<400> 14
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<210> 15
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<210> 16
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<210> 17
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<400> 17
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<210> 18
<211> 30
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<400> 18
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<210> 19
<211> 2069
<212> DNA
<213> Helicobacter pylori

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aataattaca gcaccctaaa caccctgata aaattatccg ctgatccgag cgcaattaac 180
gacgcaaggg ataacttagg ctcaagctct aggaatttgc ttgatgtcaa aaccaattcc 240
ccggcgatgc aagcgggtgt tttagcactg aatgctgcag tggggttgtg gcaagttaca 300
agctatgctt ttactgcttg tggtcctggc agtaacgaga acgcaaatgg aggtatccaa 360
actttttaata atgtgccagg acaaaatacg acgaccatca cttgcaattc gtattatgag 420
ccaggacatg gcgggcctat atccactgca aactatgcga aaatcaatca agcctatcaa 480
atcattcaaa aggctttgac agccaatgga gctaattggag atgggggtccc tgttttaagc 540
aacaccacta caaaacttga tttcactatc aatggagaca aaagaacggg gggcaaacca 600

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|            |             |            |             |             |             |      |
|------------|-------------|------------|-------------|-------------|-------------|------|
| aataaaccat | taatataacca | atggagtcac | gggaaagcta  | tttcaacctc  | gtggaatgac  | 660  |
| acacaagcaa | caccaacaac  | agaaaacatc | aacacagaaa  | ataacgctca  | agagctttta  | 720  |
| aagcaagcga | gcatcattat  | cactacccta | aatgaggcat  | gccc aaactt | tcagaatggt  | 780  |
| ggtagcgggt | attggcaagg  | gataagtggc | aatgggacaa  | tgtgtgggat  | gtttaagaat  | 840  |
| gaaatcagtg | ctatccaagg  | catgatcgct | aacgcgcaag  | aagctgtcgc  | gcaaagtaaa  | 900  |
| atcgtttagt | aaaacgcgca  | aaatcaaaac | aacttggaca  | ctggaaaacc  | attcaccctt  | 960  |
| acacagacgc | cagcttcgct  | caaagcatgc | tcaaaaacgc  | tcaagcccaa  | gcagagattt  | 1020 |
| taaaccaagc | cgaacaagt   | gtgaaaaact | ttgaaaaaat  | ccctagaaat  | tttgtatcag  | 1080 |
| actctttagg | ggtgtgttat  | gaagtgcaag | ggggtgagcg  | taggggcacc  | aatccagggtc | 1140 |
| aggtaacttc | taacacttgg  | ggggccgggt | gcgcctatgt  | ggggcaaacc  | ataacaaatc  | 1200 |
| taaaaaacag | catcgctcac  | tttggcactc | aagagcagca  | aatacaacga  | gccgaaaata  | 1260 |
| tcgctgacac | tctagtgaat  | ttcaaactta | gatacagcga  | attgggggaat | acttacaaca  | 1320 |
| gcatcaccac | tgcgctctcc  | aaagtcctta | acgcgcaaa   | cttgcaaaac  | gtggtgagca  | 1380 |
| aaaagaataa | ccccatatag  | ccgcaaggca | tagaaaccaa  | ttactacttg  | aatcaaaatt  | 1440 |
| cttaaccaaa | aatccaaacc  | atcaaccaag | aattaggggc  | taaccctttt  | aggaaagtgg  | 1500 |
| gcatcgctcg | ctctcaaacc  | aacaacggcg | ccatgaatgg  | gatcggtatt  | cagggtgggct | 1560 |
| acaagcaatt | ctttggccaa  | aaaagaaaat | ggggcgctag  | gtattacggc  | ttttttgatt  | 1620 |
| acaaccatgc | gtttattaaa  | tccagcttct | tcaactcggc  | ttctgatgtg  | tggacttatg  | 1680 |
| gttttgaggc | ggacgctctc  | tataacttca | tcaacgataa  | agccactaac  | tttttaggca  | 1740 |
| aaaacaacaa | gctttctgtg  | gggctttttg | gcgggattgc  | gttagcgggc  | acttcatggc  | 1800 |
| ttaattctga | gtatgtgaat  | ttagccaccg | tgaataatgt  | ctataacgct  | aaaatgaacg  | 1860 |
| tggcgaaact | ccaattctta  | ttcaacatgg | gagtgaggat  | gaatttgccc  | aggcccaaga  | 1920 |
| aaaaagacag | cgatcatgcg  | gctcagcatg | ggatttgagtt | agggttataa  | atccccacca  | 1980 |
| tcaacaccaa | ctactattcc  | tttatggggg | ctgaactcaa  | atacagaagg  | ctttatagcg  | 2040 |
| tgtatttgaa | ttatgtgttc  | gcttactaa  |             |             |             | 2069 |

&lt;210&gt; 20

&lt;211&gt; 2156

&lt;212&gt; DNA

&lt;213&gt; Helicobacter pylori

&lt;400&gt; 20

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| caaacaccaa  | aggcatccaa | gagctttcag | acaattatga  | aaagctgaac | aatcttttga  | 120  |
| atagttacag  | caccctaaac | acccttatca | aattgtccgc  | tgatccgagc | gcggtcagtg  | 180  |
| gcgcgatcaa  | caatttgaac | gcgggcgcga | cgggtttgct  | caaagaaaaa | accaactccc  | 240  |
| ccgcctatca  | agcgtctct  | ttggcgttaa | acgcccagct  | gggcttggtg | aataccatcg  | 300  |
| gctatgcggt  | catgtgcggg | aacgggaacg | gcacatcgaa  | tgggcctggc | agcgtgatct  | 360  |
| ttaatggaga  | gccaggacag | ggctccacgc | agattacttg  | caaccgcttt | gaatcaactg  | 420  |
| gtcctggtea  | cagcatgtct | attgatgaat | tcaagaaact  | caatcaagcc | tatcaaacta  | 480  |
| tccaacaagc  | tttaaaagga | aatgggtttc | ctgaactagg  | caacaacggc | acaagcgtga  | 540  |
| gtgttgataa  | taaatatgaa | tgcaaacaga | ataataatga  | tatcgacggc | ggtgtgagac  | 600  |
| aattctgcca  | agcgaaaaat | ggtagtagca | gtggtagtaa  | tggcagtagt | ggtaatagca  | 660  |
| agcaacaac   | cagcaagac  | ggcgtaacga | tcaccactac  | taatagtagt | aacgaagcca  | 720  |
| ccgtcaattt  | tagcatcacc | aataacgctg | aacagctggt  | aaatcaagcg | gcaaactatca | 780  |
| tgcaagtcct  | taacacgcaa | tgcccttttg | tgcatctac   | gaatgatgaa | aacgctccag  | 840  |
| ggggtggcca  | accatggggg | ttaagcacga | caggggaatgc | gtgccaaatc | ttccaacaag  | 900  |
| aatttagcca  | ggttactagc | atgatcaaaa | acgcccgaag  | aatcgtcgcg | caaagccaaa  | 960  |
| tcgctaacgc  | taaccaaaaa | gcagaaataa | ctaaccacag  | taacttcaac | cctttcacgg  | 1020 |
| acgctggcct  | tgcaaaaaac | atgctcaaaa | acgctagagc  | gcaagcagag | atgttcaatt  | 1080 |
| tagctgagca  | agtgaaaaag | aatttggaag | tcatgcaaaa  | caacaataac | gttaatgcgg  | 1140 |
| aattctacgg  | atttggggat | ggaatgacca | attttgttag  | cgcctttttg | gcaagctgca  | 1200 |
| aaaatgatgg  | gacattgcct | aatcaagggg | ttacttctaa  | cacttggggg | gccggttgcg  | 1260 |
| cgtatgtggg  | agagacgata | acggctttta | ataacagcat  | cgctcacttt | ggcactcaag  | 1320 |
| ccgagcagat  | agaacaagcc | gaaaacatcg | ctgacactct  | agtgcatttc | aaatctagat  | 1380 |
| acaatgaatt  | gggcaacact | tataacagca | tcaccaccgc  | gctctccaaa | gtccctaacg  | 1440 |
| cgcaaaagctt | gcaaaatgtg | atgggaaaaa | agaataaacc  | ctatagccca | caaggcatag  | 1500 |

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aaaccaatta ctatctcaat caaaactctt acaaccaaat ccaaaccatc aaccaagaat 1560
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<210> 21

<211> 689

<212> PRT

<213> Helicobacter pylori

<400> 21

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Tyr Glu Lys Leu Asn Asn Leu Leu Asn Asn Tyr Ser Thr Leu Asn Thr
          35          40          45
Leu Ile Lys Leu Ser Ala Asp Pro Ser Ala Ile Asn Asp Ala Arg Asp
          50          55          60
Asn Leu Gly Ser Ser Ser Arg Asn Leu Leu Asp Val Lys Thr Asn Ser
65          70          75          80
Pro Ala Tyr Gln Ala Val Leu Leu Ala Leu Asn Ala Ala Val Gly Leu
          85          90          95
Trp Gln Val Thr Ser Tyr Ala Phe Thr Ala Cys Gly Pro Gly Ser Asn
          100          105          110
Glu Asn Ala Asn Gly Gly Ile Gln Thr Phe Asn Asn Val Pro Gly Gln
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Gly Pro Ile Ser Thr Ala Asn Tyr Ala Lys Ile Asn Gln Ala Tyr Gln
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Ile Ile Gln Lys Ala Leu Thr Ala Asn Gly Ala Asn Gly Asp Gly Val
          165          170          175
Pro Val Leu Ser Asn Thr Thr Thr Lys Leu Asp Phe Thr Ile Asn Gly
          180          185          190
Asp Lys Arg Thr Gly Gly Lys Pro Asn Lys Pro Leu Ile Tyr Gln Trp
          195          200          205
Ser His Gly Lys Ala Ile Ser Thr Ser Trp Asn Asp Thr Gln Ala Thr
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Pro Thr Thr Glu Asn Ile Asn Thr Glu Asn Asn Ala Gln Glu Leu Leu
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Lys Gln Ala Ser Ile Ile Ile Thr Thr Leu Asn Glu Ala Cys Pro Asn
          245          250          255
Phe Gln Asn Gly Gly Ser Gly Tyr Trp Gln Gly Ile Ser Gly Asn Gly
          260          265          270
Thr Met Cys Gly Met Phe Lys Asn Glu Ile Ser Ala Ile Gln Gly Met
          275          280          285
Ile Ala Asn Ala Gln Glu Ala Val Ala Gln Ser Lys Ile Val Ser Glu
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Asn Ala Gln Asn Gln Asn Asn Leu Asp Thr Gly Lys Pro Phe Asn Pro

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|     |     |     |     |     | 325 |     |     |     |     |     | 330 |     |     |     |     |  | 335 |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
| Gln | Ala | Glu | Ile | Leu | Asn | Gln | Ala | Glu | Gln | Val | Val | Lys | Asn | Phe | Glu |  |     |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
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| Lys | Ile | Pro | Arg | Asn | Phe | Val | Ser | Asp | Ser | Leu | Gly | Val | Cys | Tyr | Glu |  |     |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
|     |     |     |     |     | 355 |     |     |     |     |     | 360 |     |     |     |     |  | 365 |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
| Val | Gln | Gly | Gly | Glu | Arg | Arg | Gly | Thr | Asn | Pro | Gly | Gln | Val | Thr | Ser |  |     |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
|     |     |     |     |     | 370 |     |     |     |     |     | 375 |     |     |     |     |  | 380 |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
| Asn | Thr | Asn | Gly | Ala | Gly | Cys | Ala | Tyr | Val | Gly | Gln | Thr | Ile | Thr | Asn |  |     |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
| 385 |     |     |     |     |     | 390 |     |     |     |     |     | 395 |     |     |     |  |     | 400 |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
| Leu | Lys | Asn | Ser | Ile | Ala | His | Phe | Gly | Thr | Gln | Glu | Gln | Gln | Ile | Gln |  |     |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
|     |     |     |     |     | 405 |     |     |     |     |     | 410 |     |     |     |     |  | 415 |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
| Arg | Ala | Glu | Asn | Ile | Ala | Asp | Thr | Leu | Val | Asn | Phe | Lys | Ser | Arg | Tyr |  |     |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
|     |     |     |     |     | 420 |     |     |     |     |     | 425 |     |     |     |     |  | 430 |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
| Ser | Glu | Leu | Gly | Asn | Thr | Tyr | Asn | Ser | Ile | Thr | Thr | Ala | Leu | Ser | Lys |  |     |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
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| Val | Pro | Asn | Ala | Gln | Ser | Leu | Gln | Asn | Val | Val | Ser | Lys | Lys | Asn | Asn |  |     |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
|     |     |     |     |     | 450 |     |     |     |     |     | 455 |     |     |     |     |  | 460 |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
| Pro | Tyr | Ser | Pro | Gln | Gly | Ile | Glu | Thr | Asn | Tyr | Tyr | Leu | Asn | Gln | Asn |  |     |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
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| Ser | Tyr | Asn | Gln | Ile | Gln | Thr | Ile | Asn | Gln | Glu | Leu | Gly | Arg | Asn | Pro |  |     |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
|     |     |     |     |     | 485 |     |     |     |     |     | 490 |     |     |     |     |  | 495 |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
| Phe | Arg | Lys | Val | Gly | Ile | Val | Gly | Ser | Gln | Thr | Asn | Asn | Gly | Ala | Met |  |     |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
|     |     |     |     |     | 500 |     |     |     |     |     | 505 |     |     |     |     |  | 510 |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
| Asn | Gly | Ile | Gly | Ile | Gln | Val | Gly | Tyr | Lys | Gln | Phe | Phe | Gly | Gln | Lys |  |     |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
|     |     |     |     |     | 515 |     |     |     |     |     | 520 |     |     |     |     |  | 525 |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
| Arg | Lys | Trp | Gly | Ala | Arg | Tyr | Tyr | Gly | Phe | Phe | Asp | Tyr | Asn | His | Ala |  |     |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
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|     |     |     |     |     | 565 |     |     |     |     |     | 570 |     |     |     |     |  | 575 |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
| Asn | Phe | Leu | Gly | Lys | Asn | Asn | Lys | Leu | Ser | Val | Gly | Leu | Phe | Gly | Gly |  |     |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
|     |     |     |     |     | 580 |     |     |     |     |     | 585 |     |     |     |     |  | 590 |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
| Ile | Ala | Leu | Ala | Gly | Thr | Ser | Trp | Leu | Asn | Ser | Glu | Tyr | Val | Asn | Leu |  |     |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
|     |     |     |     |     | 595 |     |     |     |     |     | 600 |     |     |     |     |  | 605 |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
| Ala | Thr | Val | Asn | Asn | Val | Tyr | Asn | Ala | Lys | Met | Asn | Val | Ala | Asn | Phe |  |     |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
|     |     |     |     |     | 610 |     |     |     |     |     | 615 |     |     |     |     |  | 620 |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
| Gln | Phe | Leu | Phe | Asn | Met | Gly | Val | Arg | Met | Asn | Leu | Ala | Arg | Pro | Lys |  |     |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
| 625 |     |     |     |     |     | 630 |     |     |     |     |     | 635 |     |     |     |  |     | 640 |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
| Lys | Lys | Asp | Ser | Asp | His | Ala | Ala | Gln | His | Gly | Ile | Glu | Leu | Gly | Leu |  |     |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
|     |     |     |     |     | 645 |     |     |     |     |     | 650 |     |     |     |     |  | 655 |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
| Lys | Ile | Pro | Thr | Ile | Asn | Thr | Asn | Tyr | Tyr | Ser | Phe | Met | Gly | Ala | Glu |  |     |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
|     |     |     |     |     | 660 |     |     |     |     |     | 665 |     |     |     |     |  | 670 |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |
| Leu | Lys | Tyr | Arg | Arg | Leu | Tyr | Ser | Val | Tyr | Leu | Asn | Tyr | Val | Phe | Ala |  |     |     |  |  |  |     |  |  |  |  |  |  |  |  |  |  |     |

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<110> American Cyanamid Company  
 Fulginiti, James P.  
 Fiske, Michael J.  
 Dilts, Deborah A.

<120> NOVEL ANTIGENS OF HELICOBACTER PYLORI

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| Met | Lys | Lys | Thr | Leu | Leu | Leu | Ser | Leu | Ser | Leu | Ser | Leu | Ser | Phe | Leu |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |
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|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
| Gly | Glu | Ala | Ala | Gln | Met | Val | Lys | Asn | Thr | Lys | Gly | Ile | Gln | Glu | Leu |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |
| Ser | Asp | Asn | Tyr | Glu | Lys | Leu | Asn | Asn | Leu | Leu | Asn | Asn | Tyr | Ser | Thr |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |
| Leu | Asn | Thr | Leu | Ile | Lys | Leu | Ser | Ala | Asp | Pro | Ser | Ala | Ile | Asn | Asp |
| 65  |     |     |     | 70  |     |     |     |     |     | 75  |     |     |     | 80  |     |
| Ala | Arg | Asp | Asn | Leu | Gly | Ser | Ser | Ser | Arg | Asn | Leu | Leu | Asp | Val | Lys |
|     |     |     | 85  |     |     |     |     |     | 90  |     |     |     |     | 95  |     |
| Thr | Asn | Ser | Pro | Ala | Tyr | Gln | Ala | Val | Leu | Leu | Ala | Leu | Asn | Ala | Ala |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
| Val | Gly | Leu | Trp | Gln | Val | Thr | Ser | Tyr | Ala | Phe | Thr | Ala | Cys | Gly | Pro |
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|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
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| 145 |     |     |     | 150 |     |     |     |     |     | 155 |     |     |     | 160 |     |
| Gly | His | Gly | Gly | Pro | Ile | Ser | Thr | Ala | Asn | Tyr | Ala | Lys | Ile | Asn | Gln |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |
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|     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |  |  |
| Ala | Tyr | Gln | Ile | Ile | Gln | Lys | Ala | Leu | Thr | Ala | Asn | Gly | Ala | Asn | Gly |  |  |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |  |  |
| Asp | Gly | Val | Pro | Val | Leu | Ser | Asn | Thr | Thr | Thr | Lys | Leu | Asp | Phe | Thr |  |  |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |  |  |
| Ile | Asn | Gly | Asp | Lys | Arg | Thr | Gly | Gly | Lys | Pro | Asn | Thr | Pro | Glu | Lys |  |  |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |  |  |
| Phe | Pro | Trp | Ser | Asp | Gly | Lys | Tyr | Ile | His | Thr | Gln | Trp | Ile | Asn | Thr |  |  |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |  |  |
| Ile | Val | Thr | Pro | Thr | Glu | Thr | Asn | Ile | Asn | Thr | Glu | Asn | Asn | Ala | Gln |  |  |
|     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |  |  |
| Glu | Leu | Leu | Lys | Gln | Ala | Ser | Ile | Ile | Ile | Thr | Thr | Leu | Asn | Glu | Ala |  |  |
|     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |  |  |
| Cys | Pro | Asn | Phe | Gln | Asn | Gly | Gly | Arg | Ser | Tyr | Trp | Gln | Gly | Ile | Ser |  |  |
|     |     | 275 |     |     |     | 280 |     |     |     |     |     | 285 |     |     |     |  |  |
| Gly | Asn | Gly | Thr | Met | Cys | Gly | Met | Phe | Lys | Asn | Glu | Ile | Ser | Ala | Ile |  |  |
|     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |  |  |
| Gln | Gly | Met | Ile | Ala | Asn | Ala | Gln | Glu | Ala | Val | Ala | Gln | Ser | Lys | Ile |  |  |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |  |  |
| Val | Ser | Glu | Asn | Ala | Gln | Asn | Gln | Asn | Asn | Leu | Asp | Thr | Gly | Lys | Pro |  |  |
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| Phe | Asn | Pro | Tyr | Thr | Asp | Ala | Ser | Phe | Ala | Gln | Ser | Met | Leu | Lys | Asn |  |  |
|     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |  |  |
| Ala | Gln | Ala | Gln | Ala | Glu | Ile | Leu | Asn | Gln | Ala | Glu | Gln | Val | Val | Lys |  |  |
|     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |  |  |
| Asn | Phe | Glu | Lys | Ile | Pro | Thr | Ala | Phe | Val | Ser | Asp | Ser | Leu | Gly | Val |  |  |
|     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |  |  |
| Cys | Tyr | Glu | Val | Gln | Gly | Gly | Glu | Arg | Arg | Gly | Thr | Asn | Pro | Gly | Gln |  |  |
| 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     | 400 |  |  |
| Val | Thr | Ser | Asn | Thr | Trp | Gly | Ala | Gly | Cys | Ala | Tyr | Val | Lys | Gln | Thr |  |  |
|     |     |     |     | 405 |     |     |     | 410 |     |     |     |     |     | 415 |     |  |  |
| Ile | Thr | Asn | Leu | Asp | Asn | Ser | Ile | Ala | His | Phe | Gly | Thr | Gln | Glu | Gln |  |  |
|     |     | 420 |     |     |     |     |     | 425 |     |     |     |     | 430 |     |     |  |  |
| Gln | Ile | Gln | Gln | Ala | Glu | Asn | Ile | Ala | Asp | Thr | Leu | Val | Asn | Phe | Lys |  |  |
|     |     | 435 |     |     |     |     | 440 |     |     |     |     | 445 |     |     |     |  |  |
| Ser | Arg | Tyr | Ser | Glu | Leu | Gly | Asn | Thr | Tyr | Asn | Ser | Ile | Thr | Thr | Ala |  |  |
|     | 450 |     |     |     |     | 455 |     |     |     |     | 460 |     |     |     |     |  |  |
| Leu | Ser | Lys | Val | Pro | Asn | Ala | Gln | Ser | Leu | Gln | Asn | Val | Val | Ser | Lys |  |  |
| 465 |     |     |     |     | 470 |     |     |     |     | 475 |     |     |     |     | 480 |  |  |
| Lys | Asn | Asn | Pro | Tyr | Ser | Pro | Gln | Gly | Ile | Glu | Thr | Asn | Tyr | Tyr | Leu |  |  |
|     |     |     |     | 485 |     |     |     | 490 |     |     |     |     |     | 495 |     |  |  |
| Asn | Gln | Asn | Ser | Tyr | Asn | Gln | Ile | Gln | Thr | Ile | Asn | Gln | Glu | Leu | Gly |  |  |
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| Arg | Asn | Pro | Phe | Arg | Lys | Val | Gly | Ile | Val | Asn | Ser | Gln | Thr | Asn | Asn |  |  |
|     |     | 515 |     |     |     |     | 520 |     |     |     |     | 525 |     |     |     |  |  |
| Gly | Ala | Met | Asn | Gly | Ile | Gly | Ile | Gln | Val | Gly | Tyr | Lys | Gln | Phe | Phe |  |  |
|     | 530 |     |     |     |     | 535 |     |     |     |     | 540 |     |     |     |     |  |  |
| Gly | Gln | Lys | Arg | Lys | Trp | Gly | Ala | Arg | Tyr | Tyr | Gly | Phe | Phe | Asp | Tyr |  |  |
| 545 |     |     |     |     | 550 |     |     |     |     | 555 |     |     |     |     | 560 |  |  |
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|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|-----------|------------|------------|------------|
|            | <400> 2    |            |            |            |            |            |            |            |            |            |            |           |            |            |            |
| Met<br>1   | Lys        | Lys        | His        | Ile<br>5   | Leu        | Ser        | Leu        | Ala        | Leu<br>10  | Gly        | Ser        | Leu       | Leu        | Val<br>15  | Ser        |
| Thr        | Leu        | Ser        | Ala<br>20  | Glu        | Asp        | Asp        | Gly        | Phe<br>25  | Tyr        | Thr        | Ser        | Val       | Gly<br>30  | Tyr        | Gln        |
| Ile        | Gly        | Glu<br>35  | Ala        | Ala        | Gln        | Met        | Val<br>40  | Thr        | Asn        | Thr        | Lys        | Gly<br>45 | Ile        | Gln        | Asp        |
| Leu        | Ser<br>50  | Asp        | Arg        | Tyr        | Glu        | Ser<br>55  | Leu        | Asn        | Asn        | Leu        | Leu<br>60  | Thr       | Arg        | Tyr        | Ser        |
| Thr<br>65  | Leu        | Asn        | Thr        | Leu        | Ile<br>70  | Lys        | Leu        | Ser        | Ala        | Asp<br>75  | Pro        | Ser       | Ala        | Ile        | Asn<br>80  |
| Ala        | Ala        | Arg        | Glu        | Asn<br>85  | Leu        | Gly        | Ala        | Ser        | Ala<br>90  | Lys        | Asn        | Leu       | Ile        | Gly<br>95  | Asp        |
| Lys        | Ala        | Asn<br>100 | Ser        | Pro        | Ala        | Tyr        | Gln        | Ala<br>105 | Val        | Leu        | Leu        | Ala       | Ile<br>110 | Asn        | Ala        |
| Ala        | Val        | Gly<br>115 | Phe        | Trp        | Asn        | Val        | Leu<br>120 | Gly        | Tyr        | Ala        | Thr        | Gln       | Cys<br>125 | Gly        | Gly        |
| Asn        | Ala<br>130 | Asn        | Gly        | Gln        | Lys        | Ser<br>135 | Thr        | Ser        | Ser        | Thr        | Thr<br>140 | Ile       | Phe        | Asn        | Asn        |
| Glu<br>145 | Pro        | Gly        | Tyr        | Arg        | Ser<br>150 | Thr        | Ser        | Ile        | Thr        | Cys<br>155 | Ser        | Leu       | Asn        | Gly        | Tyr<br>160 |
| Thr        | Pro        | Gly        | Tyr        | Tyr<br>165 | Gly        | Pro        | Met        | Ser        | Ile<br>170 | Glu        | Asn        | Phe       | Lys        | Lys<br>175 | Leu        |
| Asn        | Glu        | Ala        | Tyr<br>180 | Gln        | Ile        | Leu        | Gln        | Thr        | Ala<br>185 | Leu        | Lys        | Gln       | Gly<br>190 | Leu        | Pro        |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Leu | Lys | Glu | Asn | Asn | Lys | Lys | Val | Asn | Val | Thr | Tyr | Thr | Tyr | Thr |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
| Cys | Ser | Gly | Gly | Gly | Asn | Asn | Asn | Cys | Ser | Ser | Glu | Ala | Thr | Gly | Val |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |
| Ser | Asn | Gln | Asn | Gly | Gly | Thr | Lys | Thr | Thr | Thr | Gln | Thr | Ile | Asp | Gly |
| 225 |     |     |     |     | 230 |     |     |     |     |     | 235 |     |     |     | 240 |
| Lys | Ser | Val | Thr | Thr | Thr | Ile | Ser | Ser | Lys | Val | Val | Asp | Ser | Thr | Ala |
|     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |
| Ser | Gly | Asn | Thr | Ser | Arg | Val | Ser | Tyr | Thr | Glu | Ile | Thr | Asn | Lys | Leu |
|     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |
| Glu | Gly | Val | Pro | Asp | Ser | Ala | Gln | Ala | Leu | Leu | Ala | Gln | Ala | Ser | Thr |
|     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |
| Leu | Ile | Ser | Thr | Ile | Asn | Thr | Ala | Cys | Pro | Phe | Phe | Ser | Val | Thr | Asn |
|     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |
| Gln | Ser | Gly | Gly | Pro | Gln | Met | Glu | Pro | Thr | Lys | Gly | Lys | Leu | Cys | Gly |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |
| Phe | Thr | Glu | Glu | Ile | Ser | Ala | Ile | Gln | Lys | Met | Ile | Thr | Asp | Ala | Gln |
|     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |
| Glu | Leu | Val | Asn | Gln | Thr | Ser | Val | Ile | Asn | Ser | His | Glu | Gln | Ser | Thr |
|     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |
| Leu | Val | Gly | Gly | Asn | Asn | Gly | Lys | Pro | Phe | Asn | Pro | Phe | Thr | Asp | Ala |
|     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |
| Gln | Phe | Ala | Gln | Gly | Met | Leu | Ala | Asn | Ala | Ser | Ala | Gln | Ala | Lys | Met |
|     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |
| Leu | Asn | Leu | Ala | His | Gln | Val | Gly | Gln | Thr | Ile | Asn | Pro | Asn | Asn | Leu |
| 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     | 400 |
| Thr | Gly | Asn | Phe | Lys | Asn | Phe | Val | Thr | Gly | Phe | Leu | Ala | Thr | Cys | Asn |
|     |     |     |     | 405 |     |     |     |     | 410 |     |     |     |     | 415 |     |
| Asn | Pro | Ser | Thr | Ala | Gly | Thr | Gly | Gly | Thr | Gln | Gly | Ser | Ala | Pro | Gly |
|     |     |     | 420 |     |     |     |     | 425 |     |     |     |     | 430 |     |     |
| Thr | Val | Thr | Thr | Gln | Thr | Phe | Ala | Ser | Gly | Cys | Ala | Tyr | Val | Glu | Gln |
|     |     | 435 |     |     |     |     | 440 |     |     |     |     | 445 |     |     |     |
| Thr | Ile | Thr | Asn | Leu | Glu | Asn | Ser | Ile | Ala | His | Phe | Gly | Thr | Gln | Glu |
|     | 450 |     |     |     |     | 455 |     |     |     |     | 460 |     |     |     |     |
| Gln | Gln | Ile | Gln | Arg | Ala | Glu | Asn | Ile | Ala | Asp | Thr | Leu | Val | Asn | Phe |
| 465 |     |     |     |     | 470 |     |     |     |     | 475 |     |     |     |     | 480 |
| Lys | Ser | Arg | Tyr | Ser | Glu | Leu | Gly | Asn | Thr | Tyr | Asn | Ser | Ile | Thr | Thr |
|     |     |     |     | 485 |     |     |     |     | 490 |     |     |     |     | 495 |     |
| Ala | Leu | Ser | Lys | Val | Pro | Asn | Ala | Gln | Ser | Leu | Gln | Asn | Val | Val | Ser |
|     |     |     | 500 |     |     |     |     | 505 |     |     |     |     | 510 |     |     |
| Lys | Lys | Asn | Asn | Pro | Tyr | Ser | Pro | Gln | Gly | Ile | Glu | Thr | Asn | Tyr | Tyr |
|     |     | 515 |     |     |     |     | 520 |     |     |     |     | 525 |     |     |     |
| Leu | Asn | Gln | Asn | Ser | Tyr | Asn | Gln | Ile | Gln | Thr | Ile | Asn | Gln | Glu | Leu |
|     | 530 |     |     |     |     | 535 |     |     |     |     | 540 |     |     |     |     |
| Gly | Arg | Asn | Pro | Phe | Arg | Lys | Val | Gly | Ile | Val | Gly | Ser | Gln | Thr | Asn |
| 545 |     |     |     |     | 550 |     |     |     |     | 555 |     |     |     |     | 560 |
| Asn | Gly | Ala | Met | Asn | Gly | Ile | Gly | Ile | Gln | Val | Gly | Tyr | Glu | Gln | Phe |
|     |     |     |     | 565 |     |     |     |     | 570 |     |     |     |     | 575 |     |
| Phe | Gly | Gln | Lys | Arg | Lys | Trp | Gly | Ala | Arg | Tyr | Tyr | Gly | Phe | Phe | Asp |
|     |     |     | 580 |     |     |     |     | 585 |     |     |     |     | 590 |     |     |





|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|     |     |     | 180 |     |     |     |     | 185 |     |     |     | 190 |     |     |     |
| Glu | Leu | Gly | Gly | Asn | Gly | Thr | Lys | Val | Ser | Val | Asn | Tyr | Asn | Tyr | Glu |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
| Cys | Arg | Gln | Thr | Ala | Asp | Ile | Asn | Gly | Gly | Val | Tyr | Gln | Phe | Cys | Lys |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |
| Ala | Lys | Asn | Gly | Ser | Ser | Ser | Ser | Ser | Asn | Gly | Gly | Asn | Gly | Ser | Ser |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |
| Thr | Gln | Thr | Thr | Ala | Thr | Thr | Thr | Gln | Asp | Gly | Val | Thr | Ile | Thr | Thr |
|     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |
| Thr | Tyr | Asn | Asn | Asn | Lys | Ala | Thr | Val | Lys | Phe | Asp | Ile | Thr | Asn | Asn |
|     |     |     | 260 |     |     |     |     | 265 |     |     |     | 270 |     |     |     |
| Ala | Glu | Gln | Leu | Leu | Asn | Gln | Ala | Ala | Asn | Ile | Met | Gln | Val | Leu | Asn |
|     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |
| Thr | Gln | Cys | Pro | Leu | Val | Arg | Ser | Thr | Asn | Asn | Glu | Asn | Thr | Pro | Gly |
|     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |
| Gly | Gly | Gln | Pro | Trp | Gly | Leu | Ser | Thr | Ser | Gly | Asn | Ala | Cys | Ser | Ile |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |
| Phe | Gln | Gln | Glu | Phe | Ser | Gln | Val | Thr | Ser | Met | Ile | Lys | Asn | Ala | Gln |
|     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |
| Glu | Ile | Ile | Ala | Gln | Ser | Lys | Ile | Val | Ser | Glu | Asn | Ala | Gln | Asn | Gln |
|     |     |     | 340 |     |     |     |     | 345 |     |     |     | 350 |     |     |     |
| Asn | Asn | Leu | Asp | Thr | Gly | Lys | Pro | Phe | Asn | Pro | Tyr | Thr | Asp | Ala | Ser |
|     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |
| Phe | Ala | Gln | Ser | Met | Leu | Lys | Asn | Ala | Gln | Ala | Gln | Ala | Glu | Met | Phe |
|     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |
| Asn | Leu | Ser | Glu | Gln | Val | Lys | Lys | Asn | Leu | Glu | Val | Met | Lys | Asn | Asn |
| 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     | 400 |
| Asn | Asn | Val | Asn | Glu | Lys | Leu | Ala | Gly | Phe | Gly | Lys | Glu | Glu | Val | Met |
|     |     |     | 405 |     |     |     |     | 410 |     |     |     |     |     | 415 |     |
| Thr | Asn | Phe | Val | Ser | Ala | Phe | Leu | Ala | Ser | Cys | Lys | Asp | Gly | Gly | Thr |
|     |     |     | 420 |     |     |     |     | 425 |     |     |     |     | 430 |     |     |
| Leu | Pro | Asn | Ala | Gly | Val | Thr | Ser | Asn | Thr | Trp | Gly | Ala | Gly | Cys | Ala |
|     |     | 435 |     |     |     |     | 440 |     |     |     |     | 445 |     |     |     |
| Tyr | Val | Gly | Glu | Thr | Ile | Ser | Ala | Leu | Thr | Asn | Ser | Ile | Ala | His | Phe |
|     | 450 |     |     |     |     | 455 |     |     |     |     | 460 |     |     |     |     |
| Gly | Thr | Gln | Glu | Gln | Gln | Ile | Gln | Gln | Ala | Glu | Asn | Ile | Ala | Asp | Thr |
| 465 |     |     |     |     | 470 |     |     |     |     | 475 |     |     |     |     | 480 |
| Leu | Val | Asn | Phe | Lys | Ser | Arg | Tyr | Ser | Glu | Leu | Gly | Asn | Thr | Tyr | Asn |
|     |     |     | 485 |     |     |     |     |     | 490 |     |     |     |     | 495 |     |
| Ser | Ile | Thr | Thr | Ala | Leu | Ser | Lys | Val | Pro | Asn | Ala | Gln | Ser | Leu | Gln |
|     |     |     | 500 |     |     |     |     | 505 |     |     |     | 510 |     |     |     |
| Asn | Val | Val | Ser | Lys | Lys | Asn | Asn | Pro | Tyr | Ser | Pro | Gln | Gly | Ile | Glu |
|     |     | 515 |     |     |     |     | 520 |     |     |     |     | 525 |     |     |     |
| Thr | Asn | Tyr | Tyr | Leu | Asn | Gln | Asn | Ser | Tyr | Asn | Gln | Ile | Gln | Thr | Ile |
|     | 530 |     |     |     |     | 535 |     |     |     |     | 540 |     |     |     |     |
| Asn | Gln | Glu | Leu | Gly | Arg | Asn | Pro | Phe | Arg | Lys | Val | Gly | Ile | Val | Asn |
| 545 |     |     |     |     | 550 |     |     |     |     | 555 |     |     |     |     | 560 |
| Ser | Gln | Thr | Asn | Asn | Gly | Ala | Met | Asn | Gly | Ile | Gly | Ile | Gln | Val | Gly |
|     |     |     | 565 |     |     |     |     |     | 570 |     |     |     |     | 575 |     |
| Tyr | Lys | Gln | Phe | Phe | Gly | Gln | Lys | Arg | Lys | Trp | Gly | Ala | Arg | Tyr | Tyr |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |  |     |  |  |  |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|--|--|-----|--|--|--|--|
| 580 |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 585 |  |  |  |  | 590 |  |  |  |  |
| Gly | Phe | Phe | Asp | Tyr | Asn | His | Ala | Phe | Ile | Lys | Ser | Ser | Phe | Phe | Asn |  |  |  |  |     |  |  |  |  |
|     |     |     | 595 |     |     |     | 600 |     |     |     |     | 605 |     |     |     |  |  |  |  |     |  |  |  |  |
| Ser | Ala | Ser | Asp | Val | Trp | Thr | Tyr | Gly | Phe | Gly | Ala | Asp | Ala | Leu | Tyr |  |  |  |  |     |  |  |  |  |
|     |     |     | 610 |     |     |     | 615 |     |     |     |     | 620 |     |     |     |  |  |  |  |     |  |  |  |  |
| Asn | Phe | Ile | Asn | Asp | Lys | Ala | Thr | Asn | Phe | Leu | Gly | Lys | Asn | Asn | Lys |  |  |  |  |     |  |  |  |  |
| 625 |     |     |     |     | 630 |     |     |     |     |     | 635 |     |     |     | 640 |  |  |  |  |     |  |  |  |  |
| Leu | Ser | Leu | Gly | Leu | Phe | Gly | Gly | Ile | Ala | Leu | Ala | Gly | Thr | Ser | Trp |  |  |  |  |     |  |  |  |  |
|     |     |     |     | 645 |     |     |     |     |     | 650 |     |     |     |     | 655 |  |  |  |  |     |  |  |  |  |
| Leu | Asn | Ser | Glu | Tyr | Val | Asn | Leu | Ala | Thr | Val | Asn | Asn | Val | Tyr | Asn |  |  |  |  |     |  |  |  |  |
|     |     |     | 660 |     |     |     |     | 665 |     |     |     |     | 670 |     |     |  |  |  |  |     |  |  |  |  |
| Ala | Lys | Met | Asn | Val | Ala | Asn | Phe | Gln | Phe | Leu | Phe | Asn | Met | Gly | Val |  |  |  |  |     |  |  |  |  |
|     |     |     | 675 |     |     |     | 680 |     |     |     |     |     | 685 |     |     |  |  |  |  |     |  |  |  |  |
| Arg | Met | Asn | Leu | Ala | Arg | Ser | Lys | Lys | Lys | Gly | Ser | Asp | His | Ala | Ala |  |  |  |  |     |  |  |  |  |
|     |     |     | 690 |     |     | 695 |     |     |     |     |     | 700 |     |     |     |  |  |  |  |     |  |  |  |  |
| Gln | His | Gly | Ile | Glu | Leu | Gly | Leu | Lys | Ile | Pro | Thr | Ile | Asn | Thr | Asn |  |  |  |  |     |  |  |  |  |
| 705 |     |     |     |     | 710 |     |     |     |     |     | 715 |     |     |     | 720 |  |  |  |  |     |  |  |  |  |
| Tyr | Tyr | Ser | Phe | Met | Gly | Ala | Glu | Leu | Lys | Tyr | Arg | Arg | Leu | Tyr | Ser |  |  |  |  |     |  |  |  |  |
|     |     |     |     | 725 |     |     |     |     |     | 730 |     |     |     |     | 735 |  |  |  |  |     |  |  |  |  |
| Val | Tyr | Leu | Asn | Tyr | Val | Phe | Ala | Tyr |     |     |     |     |     |     |     |  |  |  |  |     |  |  |  |  |
|     |     |     | 740 |     |     |     |     | 745 |     |     |     |     |     |     |     |  |  |  |  |     |  |  |  |  |

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<211> 2127
<212> DNA
<213> Helicobacter pylori
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| gacgacggct  | tttacacaag | cgtgggctat  | caaatcggtg | aagccgctca  | aatggtgaaa  | 120  |
| aacaccaaag  | gcattcaaga | gcttttcagac | aattatgaaa | agctgaacaa  | tcttttgaat  | 180  |
| aattacagca  | ccctaaacac | ccttatcaaa  | ttgtccgctg | atccgagcgc  | gattaacgac  | 240  |
| gcaagggata  | atctaggctc | aagctctagg  | aatttgcttg | atgtcaaaac  | caattccccc  | 300  |
| gcgtatcaag  | ccgtgctttt | agcactcaat  | gctgcagtgg | ggttgtggca  | agttacaagc  | 360  |
| tacgctttta  | ctgcttggtg | tcctggcagt  | aacgagaatg | cgaatggagg  | gatccaaact  | 420  |
| tttaataatg  | tgccaggaca | agatacgacg  | accatcactt | gcaattcgtg  | ttatgagcca  | 480  |
| ggacatggtg  | ggcctatatc | cactgcaa    | tatgcgaaaa | tcaatcaagc  | ctatcaa     | 540  |
| atccaaaagg  | ctttgacagc | caatggagct  | aatggagatg | gggtccccgt  | tttaagcaac  | 600  |
| accactacaa  | aacttgattt | cactatcaat  | ggagacaaaa | gaacgggggg  | caaacc      | 660  |
| acacctgaaa  | agttcccatg | gagtgatggg  | aaatatattc | acacc       | caatg       | 720  |
| atagtaacac  | caacagaaac | aaatatcaac  | acagaaaata | acgctcaaga  | gcttttaaaa  | 780  |
| caagcgagca  | tcattatcac | taccctaaat  | gaggcatgcc | caaacttcca  | aatggtggt   | 840  |
| agaagttatt  | ggcaagggat | aagcggcaat  | gggacaatgt | gcgggatgtt  | taagaatgaa  | 900  |
| atcagcgcga  | tccaaggcat | gatcgctaac  | gctcaagaag | ctgtcgcgca  | aagcaaaatc  | 960  |
| gttagtgaaa  | acgcgcaaaa | tcaaaacaac  | ttggatactg | gaaaaccatt  | caacccttac  | 1020 |
| acggacgcca  | gctttgcgca | aagcatgctc  | aaaaacgctc | aagcgcaagc  | agagatttta  | 1080 |
| aaccaagccg  | aacaagtagt | aaaaaacttt  | gaaaaaatcc | ctacagcctt  | tgtatcagac  | 1140 |
| tcttttagggg | tgtgttatga | agtgcaaggg  | ggtgagcgta | ggggcaccaa  | tccagggtcag | 1200 |
| gtaacttcta  | acacttgggg | agccggttgc  | gcgtatgtga | aacaaaccat  | aacgaattta  | 1260 |
| gacaacagca  | tcgctcactt | tggcactcaa  | gagcagcaga | tacagcaagc  | cgaaaacatc  | 1320 |

|            |            |            |            |            |            |      |
|------------|------------|------------|------------|------------|------------|------|
| gctgacactc | tagtgaattt | caaatctaga | tacagcgaat | taggcaacac | ctataacagc | 1380 |
| atcaccaccg | cgctctccaa | agtcctaac  | gcgcaaagct | tgcaaacgt  | ggtgagcaaa | 1440 |
| aagaataacc | cctatagccc | tcaaggcata | gagaccaatt | actacctcaa | tcaaaattct | 1500 |
| tacaacaaaa | tccaaaccat | caaccaagaa | ctagggcgta | acccctttag | gaaagtgggc | 1560 |
| atcgtcaatt | ctcaaaccaa | caatggtgcc | atgaatggga | tcggtattca | ggtgggctat | 1620 |
| aagcaattct | ttggccaaaa | aagaaaatgg | ggcgctaggt | attacggctt | ttttgactac | 1680 |
| aaccatgcgt | tcattaaatc | cagcttcttc | aactcggctt | ctgatgtgtg | gacttatggt | 1740 |
| tttggagcgg | acgctcttta | taacttcata | aacgataaag | ccaccaattt | cttaggcaaa | 1800 |
| aacaacaagc | tttccgtggg | gctttttgga | gggattgcgt | tagcgggcac | ttcatggcct | 1860 |
| aattctgagt | atgtgaattt | agccaccgtg | aataacgtct | ataacgctaa | aatgaatgtg | 1920 |
| gcgaatttcc | aattcttatt | caatatggga | gtgaggatga | atntagccag | atccaagaaa | 1980 |
| aaaggcagcg | atcatgcggc | tcagcatggg | attgaactag | ggcttaaaat | ccccaccatc | 2040 |
| aacacgaact | attattcttt | catgggggct | gaactcaaat | acagaaggct | ttatagcgtg | 2100 |
| tatttgaatt | atgtgttcgc | ttactaa    |            |            |            | 2127 |

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<211> 2226

<212> DNA

<213> Helicobacter pylori

<400> 5

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| gaagacgacg  | gcttttacac  | aagcgtgggc  | tatcaaactg | gtgaagccgc | tcaaattggt | 120  |
| acaaacacca  | aaggcattca  | agatctttca  | gatcgttatg | aaagtttgaa | caaccttttg | 180  |
| acccgataca  | gcaccctaaa  | cacctgatc   | aaattgtccg | ctgatccgag | cgcgattaat | 240  |
| gcggcgcggtg | aaaatctggg  | cgcgagcgcg  | aagaatttga | tcggcgataa | agccaattcc | 300  |
| ccagcctatc  | aagcgggtgct | tttagcgatc  | aacgcggcgg | tagggttttg | gaatgtctta | 360  |
| ggctatgcta  | cgcaatgcgg  | gggtaacgcc  | aatggtcaaa | aaagcacctc | ttcaacgacc | 420  |
| atcttcaaca  | acgagccagg  | gtatcgatcc  | acttccatca | cttgctcttt | gaacgggtat | 480  |
| acgcctggat  | actatggccc  | tatgagtatt  | gagaatttca | aaaagcttaa | cgaagcctat | 540  |
| cagatcctcc  | aaacggcggt  | aaaacaaggc  | ttaccgcgcg | tcaaagaaaa | caacaagaag | 600  |
| gtcaatgtta  | cctacactta  | cacatgctca  | gggggaggga | ataataactg | ctcgtcagaa | 660  |
| gccacagggtg | taagcaatca  | aaatggcgga  | actaaaacca | ccacccaaac | catagaggcg | 720  |
| aaaagcgtaa  | ccaccacgat  | cagttcaaaa  | gtcgttgata | gcacagcgag | tggtaacaca | 780  |
| tcacgtgtct  | cctacaccga  | aatcaccaac  | aaattagaag | gtgtgcctga | tagcgtcaa  | 840  |
| gcgctcttag  | cgcaagcgag  | cacgctcatt  | agcaccatca | acacggcatg | cccgtttttt | 900  |
| agtgtaaact  | atcaaagtgg  | tgggtccacag | atggaaccga | ctaaagggaa | gttgtgcggt | 960  |
| tttacagaag  | aaatcagcgc  | gatccaaaag  | atgatcacag | acgcgcaaga | gctggtcaat | 1020 |
| caaacgagcg  | tcattaatag  | ccatgaacaa  | tcaaccctag | tgggcggtaa | taatggcaag | 1080 |
| cctttcaacc  | ctttcacgga  | cgctcaattc  | gctcaaggca | tgctcgctaa | cgctagcgcg | 1140 |
| caagctaaaa  | tgtctcaattt | agcccatcaa  | gtggggcaaa | ccattaaccc | taacaattct | 1200 |
| actgggaatt  | ttaaaaattt  | tgttacaggc  | tttttagcca | catgcaacaa | ccctcaaca  | 1260 |
| gctggcactg  | gtggcacaca  | aggttcagct  | ccaggcacgg | ttaccactca | aactttcgct | 1320 |
| tccggttgcg  | cgtatgtgga  | acaaaccata  | acgaatttag | aaaacagcat | cgcgcacttt | 1380 |
| ggcactcaag  | agcagcaaat  | acaacgagcc  | gaaaatatcg | ctgacactct | agtgaatttc | 1440 |
| aaatctagat  | acagcgaatt  | ggggaatact  | tacaacagca | tcaccactgc | gctctccaaa | 1500 |
| gtccctaacg  | cgcaaagctt  | gcaaaacgtg  | gtgagcaaaa | agaataaccc | ctatagcccg | 1560 |
| caaggcatag  | aaaccaatta  | ctacttgaat  | caaaattctt | acaaccaaat | ccaaaccatc | 1620 |
| aaccaagaat  | tagggcgtaa  | cccttttagg  | aaagtgggca | tcgtcggctc | tcaaaccaac | 1680 |
| aacggcgcca  | tgaatgggat  | cggtattcag  | gtgggctacg | agcaattctt | tggccaaaaa | 1740 |

|            |            |            |            |            |            |      |
|------------|------------|------------|------------|------------|------------|------|
| agaaaatggg | gcgctaggta | ttacggcttt | tttgattaca | accatgcggt | tattaaatcc | 1800 |
| agcttcttca | actcggtctc | tgatgtgtgg | acttatgggt | ttggagcgga | cgctctctat | 1860 |
| aacttcatca | acgataaagc | cactaacttt | ttaggcaaaa | acaacaagct | ttctgtgggg | 1920 |
| ctttttggcg | ggattgctgt | agcgggcact | tcatggctta | attctgagta | tgtgaattta | 1980 |
| gccacgtga  | ataatgtcta | taacgctaaa | atgaacgtgg | cgaacttcca | attcttattc | 2040 |
| aacatgggag | tgaggatgaa | tttggccagg | ccaagaaaa  | acgacagcga | tcatgcggct | 2100 |
| cagcatggga | ttgagttagg | gcttaaaatc | cccaccatca | acacgaacta | ctattccttt | 2160 |
| atgggggctg | aactcaaata | cagaaggctt | tatagcgtgt | atttgaatta | tgtgttcgct | 2220 |
| tactag     |            |            |            |            |            | 2226 |

&lt;210&gt; 6

&lt;211&gt; 2238

&lt;212&gt; DNA

&lt;213&gt; Helicobacter pylori

&lt;400&gt; 6

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| gaagacgacg  | gcttttacac  | aagcgtaggc | tatcagatcg | gtgaagccgc  | tcaaatggta | 120  |
| acaaacacca  | aaggcatcca  | acagctttca | gacaattatg | aaaatttgaa  | caacctttta | 180  |
| acgagataca  | gcaccctaaa  | cacccttata | aaattgtccg | ctgatccgag  | cgcaattaat | 240  |
| gcggtgcggg  | aaaatctggg  | cgcgagcacg | aagaatttga | tggcgataaa  | agccaactcc | 300  |
| ccggcgatatc | aagccgtgtt  | tttagcgatc | aacgcggcgg | tagggttgtg  | gaataccatc | 360  |
| ggctatgcgg  | tcatgtgcgg  | gaacgggaac | ggcacagaga | gtgggcctgg  | cagcgtgatc | 420  |
| tttaatgacc  | aaccaggaca  | ggattccacg | caaattactt | gcaaccgctt  | tgaatcaact | 480  |
| gggcctggta  | aaagcatgtc  | tattgatgaa | ttcaaaaaac | tcaatgaagc  | ctatcaaatc | 540  |
| atccagcaag  | ctttaaaaaa  | tcaaagtggg | tttcttgaat | taggcgggaa  | cggcacaaaa | 600  |
| gtgagtgtta  | attacaatta  | cgaatgcaga | caaactgctg | atatcaacgg  | cgggtgtgat | 660  |
| cagttctgca  | aggctaaaaa  | tggtagtagt | agcagtagta | atggcggtaa  | tggcagtagc | 720  |
| acgcaaacaa  | ccgcgacaac  | cacgcaagac | ggcgtaacga | tcaccactac  | ctataataat | 780  |
| aacaaagcca  | ccgtcaaatt  | tgacatcacc | aataacgctg | aacagctggt  | aatcaagcg  | 840  |
| gcaaacatca  | tgcaagtcc   | taatacgcaa | tgccctttag | tgcgttccac  | gaataacgaa | 900  |
| aacactccag  | ggggtggtca  | accatggggg | ttaagcacat | ccgggaatgc  | gtgcagcatc | 960  |
| ttccaacaag  | aatttagcca  | ggttactagc | atgatcaaaa | acgcccaga   | aataatcgcg | 1020 |
| caaagcaaaa  | tcgtagtgta  | aaacgcgcaa | aatcaaaaac | acttggatac  | tggaaaacca | 1080 |
| ttcaaccctt  | acacggacgc  | cagctttgcg | caaagcatgc | tcaaaaacgc  | tcaagcgcaa | 1140 |
| gcagagatgt  | tcaatttgag  | cgaacaagtg | aaaaagaact | tggaaagtc   | gaaaaacaac | 1200 |
| aataatgtta  | acgagaaatt  | agcaggattt | gggaaagaag | aagtaatgac  | caattttgtt | 1260 |
| agcgcttttt  | tggcaagctg  | caaagatggt | ggcacattgc | ctaatagcagg | ggttacttct | 1320 |
| aacacttggtg | gggcgggttg  | cgcgtatgtg | ggagagacga | taagcgccct  | aaccaacagc | 1380 |
| atcgctcact  | ttggcactca  | agagcagcag | atacagcaag | ccgaaaacat  | cgctgacact | 1440 |
| ctagtgaatt  | tcaaatctag  | atacagcgaa | ttaggcaaca | cctataacag  | catcaccacc | 1500 |
| gcgctctcca  | aagtccctaa  | cgcgcaaagc | ttgcaaaacg | tggtagcaa   | aaagaataac | 1560 |
| ccctatagcc  | ctcaaggcat  | agagaccaat | tactacctca | atcaaaattc  | ttacaaccaa | 1620 |
| atccaaacca  | tcaaccaaga  | actagggcgt | aaccccttta | ggaaagtggg  | catcgtaaat | 1680 |
| tctcaaacca  | acaatgggtg  | catgaattgg | atcggcattc | aggtgggcta  | taagcaattc | 1740 |
| tttggccaaa  | aaagaaaattg | gggcgctagg | tattacggct | tttttgatta  | caaccatgcg | 1800 |
| ttcatcaaat  | ccagcttttt  | caactcggtc | tctgacgtgt | ggacttatgg  | ttttggagcg | 1860 |
| gacgcgtttt  | ataacttcat  | caacgataaa | gccaccaatt | tcttaggcaa  | aaacaacaag | 1920 |
| ctttcttttg  | ggcttttttg  | cgggattgcg | ttagcgggca | cttcatggct  | caattctgag | 1980 |
| tacgtgaatt  | tagccaccgt  | gaataacgtc | tataacgcta | aaatgaatgt  | ggcgaatttc | 2040 |

caattcttat tcaatatggg agtgaggatg aatttagcca gatccaagaa aaaaggcagc 2100  
 gatcatgcag ctcagcatgg gattgagtta gggcttaaaa tccccaccat caacacgaac 2160  
 tattattcct ttatgggggc tgaactcaaa tacagaaggc tctatagcgt gtatttgaac 2220  
 tatgtgttcg cttactaa 2238

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 <211> 20  
 <212> PRT  
 <213> Helicobacter pylori

<400> 7  
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 Ala Gln Met Val  
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<210> 8  
 <211> 33  
 <212> DNA  
 <213> Artificial sequence

<220>  
 <223> Forward Primer

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<210> 9  
 <211> 42  
 <212> DNA  
 <213> Artificial sequence

<220>  
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<210> 10  
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 <212> DNA  
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<220>  
 <223> Primer for promoter region of 75 kDa gene

<400> 10  
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<210> 11

<211> 33  
 <212> DNA  
 <213> Artificial sequence

<220>  
 <223> Primer for 5' start mature 75, 77, and 7kDa genes

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<210> 12  
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 <212> DNA  
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<220>  
 <223> Primer for 3' end of 75, 77, and 7kDa genes

<400> 12  
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<210> 13  
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<220>  
 <223> Primer for internal 75 kDa specific

<400> 13  
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<210> 14  
 <211> 21  
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<220>  
 <223> Primer for internal 75 kDa specific

<400> 14  
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<210> 15  
 <211> 36  
 <212> DNA  
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<220>  
 <223> Primer for internal 75 kDa seamless cloning

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<210> 16  
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<212> DNA  
<213> Artificial sequence

<220>  
<223> Primer for internal 7kDa seamless cloning

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<210> 17  
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<212> DNA  
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<223> Primer for 5' end start mature 75, 77, and 7kDa genes

<400> 17  
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<210> 18  
<211> 30  
<212> DNA  
<213> Artificial sequence

<220>  
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<400> 18  
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<212> DNA  
<213> Helicobacter pylori

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aataattaca gcaccctaaa caccctgacg aaattatccg ctgatccgag cgcaattaac 180  
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|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| atcattcaaa  | aggctttgac  | agccaatgga  | gctaattggag | atgggggtccc | tgtttttaagc | 540  |
| aacaccacta  | caaaacttga  | tttactatc   | aatggagaca  | aaagaacggg  | gggcaaacca  | 600  |
| aataaaccat  | taatatacca  | atggagtc    | gggaaagcta  | tttcaacctc  | gtggaatgac  | 660  |
| acacaagcaa  | caccaacaac  | agaaaacatc  | aacacagaaa  | ataacgctca  | agagctttta  | 720  |
| aagcaagcga  | gcattcattat | cactacccta  | aatgaggcat  | gcccaaactt  | tcagaatggg  | 780  |
| ggtagcgggt  | attggcaagg  | gataagtggc  | aatgggacaa  | tgtgtgggat  | gtttaagaat  | 840  |
| gaaatcagtg  | ctatccaagg  | catgatcgct  | aacgcgcaag  | aagctgtcgc  | gcaaagtaaa  | 900  |
| atcgtttagtg | aaaacgcgca  | aaatcaaaac  | aacttggaca  | ctggaaaacc  | attcaccctt  | 960  |
| acacagacgc  | cagcttcgct  | caaagcatgc  | tcaaaaacgc  | tcaagcccaa  | gcagagattt  | 1020 |
| taaaccaagc  | cgaacaagtg  | gtgaaaaaact | ttgaaaaaat  | ccctagaaat  | tttgtatcag  | 1080 |
| actcttttagg | gggtgtgttat | gaagtgcgaag | gggggtgagcg | taggggcacc  | aatccagggtc | 1140 |
| aggtaacttc  | taacacttgg  | ggggccgggt  | gcgcctatgt  | ggggcaaacc  | ataacaaatc  | 1200 |
| taaaaaacag  | catcgctcac  | tttggcactc  | aagagcagca  | aatacaacga  | gccgaaaata  | 1260 |
| tcgctgacac  | tctagtgaat  | ttcaaatacta | gatacagcga  | attgggggaat | acttacaaca  | 1320 |
| gcataccac   | tgcgctctcc  | aaagtcccta  | acgcgcaaag  | cttgcaaaac  | gtgggtgagca | 1380 |
| aaaagaataa  | cccctatagc  | ccgcaaggca  | tagaaaccaa  | ttactacttg  | aatcaaaatt  | 1440 |
| cttacaacca  | aatccaaacc  | atcaaccaag  | aattagggcg  | taaccctttt  | aggaaagtgg  | 1500 |
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| ttaattctga  | gtatgtgaat  | ttagccaccg  | tgaataatgt  | ctataacgct  | aaaatgaacg  | 1860 |
| tggcgaaactt | ccaattctta  | ttcaacatgg  | gagtgaggat  | gaatttgcc   | aggcccaaga  | 1920 |
| aaaaagacag  | cgatcatgcg  | gctcagcatg  | ggattgagtt  | agggcttaaa  | atccccacca  | 1980 |
| tcaacaccaa  | ctactattec  | tttatggggg  | ctgaactcaa  | atacagaagg  | cttttatagcg | 2040 |
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&lt;210&gt; 20

&lt;211&gt; 2156

&lt;212&gt; DNA

&lt;213&gt; Helicobacter pylori

&lt;400&gt; 20

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| caaacaccaa  | aggcatccaa | gagctttcag  | acaattatga  | aaagctgaac  | aatcttttga  | 120 |
| atagttacag  | caccctaaac | acccttatca  | aattgtccgc  | tgatccgagc  | gcggtcagtg  | 180 |
| gcgcgatcaa  | caatttgaac | gcgggcgcga  | cgggtttgct  | caaagaaaaa  | accaactccc  | 240 |
| ccgcctatca  | agccgtctct | ttggcggttaa | acgccgcagt  | gggcttggtg  | aataccatcg  | 300 |
| gctatgcggg  | catgtgcggg | aacgggaacg  | gcacatcgaa  | tgggcctggc  | agcgtgatct  | 360 |
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| ccgtcaattt  | tagcatcacc | aataacgctg  | aacagctggt  | aaatcaagcg  | gcaaacatca  | 780 |
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| aatttagcca  | ggttactagc | atgatcaaaa  | acgcccgaaga | aatcgctcgcg | caaagccaaa  | 960 |

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<210> 21

<211> 689

<212> PRT

<213> *Helicobacter pylori*

<400> 21

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Tyr Glu Lys Leu Asn Asn Leu Leu Asn Asn Tyr Ser Thr Leu Asn Thr
35     40     45
Leu Ile Lys Leu Ser Ala Asp Pro Ser Ala Ile Asn Asp Ala Arg Asp
50     55     60
Asn Leu Gly Ser Ser Ser Arg Asn Leu Leu Asp Val Lys Thr Asn Ser
65     70     75     80
Pro Ala Tyr Gln Ala Val Leu Leu Ala Leu Asn Ala Ala Val Gly Leu
85     90     95
Trp Gln Val Thr Ser Tyr Ala Phe Thr Ala Cys Gly Pro Gly Ser Asn
100    105    110
Glu Asn Ala Asn Gly Gly Ile Gln Thr Phe Asn Asn Val Pro Gly Gln
115    120    125
Asn Thr Thr Thr Ile Thr Cys Asn Ser Tyr Tyr Glu Pro Gly His Gly
130    135    140
Gly Pro Ile Ser Thr Ala Asn Tyr Ala Lys Ile Asn Gln Ala Tyr Gln
145    150    155    160
Ile Ile Gln Lys Ala Leu Thr Ala Asn Gly Ala Asn Gly Asp Gly Val
165    170    175
Pro Val Leu Ser Asn Thr Thr Thr Lys Leu Asp Phe Thr Ile Asn Gly

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
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|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |  |
| Asp | Lys | Arg | Thr | Gly | Gly | Lys | Pro | Asn | Lys | Pro | Leu | Ile | Tyr | Gln | Trp |  |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |  |
| Ser | His | Gly | Lys | Ala | Ile | Ser | Thr | Ser | Trp | Asn | Asp | Thr | Gln | Ala | Thr |  |
|     | 210 |     |     |     |     | 215 |     |     |     | 220 |     |     |     |     |     |  |
| Pro | Thr | Thr | Glu | Asn | Ile | Asn | Thr | Glu | Asn | Asn | Ala | Gln | Glu | Leu | Leu |  |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |  |
| Lys | Gln | Ala | Ser | Ile | Ile | Ile | Thr | Thr | Leu | Asn | Glu | Ala | Cys | Pro | Asn |  |
|     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |  |
| Phe | Gln | Asn | Gly | Gly | Ser | Gly | Tyr | Trp | Gln | Gly | Ile | Ser | Gly | Asn | Gly |  |
|     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |  |
| Thr | Met | Cys | Gly | Met | Phe | Lys | Asn | Glu | Ile | Ser | Ala | Ile | Gln | Gly | Met |  |
|     | 275 |     |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |  |
| Ile | Ala | Asn | Ala | Gln | Glu | Ala | Val | Ala | Gln | Ser | Lys | Ile | Val | Ser | Glu |  |
|     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |  |
| Asn | Ala | Gln | Asn | Gln | Asn | Asn | Leu | Asp | Thr | Gly | Lys | Pro | Phe | Asn | Pro |  |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |  |
| Tyr | Thr | Asp | Ala | Ser | Phe | Ala | Gln | Ser | Met | Leu | Lys | Asn | Ala | Gln | Ala |  |
|     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |  |
| Gln | Ala | Glu | Ile | Leu | Asn | Gln | Ala | Glu | Gln | Val | Val | Lys | Asn | Phe | Glu |  |
|     |     |     | 340 |     |     |     |     | 345 |     |     |     | 350 |     |     |     |  |
| Lys | Ile | Pro | Arg | Asn | Phe | Val | Ser | Asp | Ser | Leu | Gly | Val | Cys | Tyr | Glu |  |
|     | 355 |     |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |  |
| Val | Gln | Gly | Gly | Glu | Arg | Arg | Gly | Thr | Asn | Pro | Gly | Gln | Val | Thr | Ser |  |
|     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |  |
| Asn | Thr | Asn | Gly | Ala | Gly | Cys | Ala | Tyr | Val | Gly | Gln | Thr | Ile | Thr | Asn |  |
| 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     | 400 |  |
| Leu | Lys | Asn | Ser | Ile | Ala | His | Phe | Gly | Thr | Gln | Glu | Gln | Gln | Ile | Gln |  |
|     |     |     |     | 405 |     |     |     |     | 410 |     |     |     |     | 415 |     |  |
| Arg | Ala | Glu | Asn | Ile | Ala | Asp | Thr | Leu | Val | Asn | Phe | Lys | Ser | Arg | Tyr |  |
|     |     |     | 420 |     |     |     |     | 425 |     |     |     |     | 430 |     |     |  |
| Ser | Glu | Leu | Gly | Asn | Thr | Tyr | Asn | Ser | Ile | Thr | Thr | Ala | Leu | Ser | Lys |  |
|     | 435 |     |     |     |     |     | 440 |     |     |     |     | 445 |     |     |     |  |
| Val | Pro | Asn | Ala | Gln | Ser | Leu | Gln | Asn | Val | Val | Ser | Lys | Lys | Asn | Asn |  |
|     | 450 |     |     |     |     | 455 |     |     |     |     | 460 |     |     |     |     |  |
| Pro | Tyr | Ser | Pro | Gln | Gly | Ile | Glu | Thr | Asn | Tyr | Tyr | Leu | Asn | Gln | Asn |  |
| 465 |     |     |     |     | 470 |     |     |     |     | 475 |     |     |     |     | 480 |  |
| Ser | Tyr | Asn | Gln | Ile | Gln | Thr | Ile | Asn | Gln | Glu | Leu | Gly | Arg | Asn | Pro |  |
|     |     |     |     | 485 |     |     |     |     | 490 |     |     |     |     | 495 |     |  |
| Phe | Arg | Lys | Val | Gly | Ile | Val | Gly | Ser | Gln | Thr | Asn | Asn | Gly | Ala | Met |  |
|     |     |     | 500 |     |     |     |     | 505 |     |     |     |     | 510 |     |     |  |
| Asn | Gly | Ile | Gly | Ile | Gln | Val | Gly | Tyr | Lys | Gln | Phe | Phe | Gly | Gln | Lys |  |
|     | 515 |     |     |     |     |     | 520 |     |     |     |     | 525 |     |     |     |  |
| Arg | Lys | Trp | Gly | Ala | Arg | Tyr | Tyr | Gly | Phe | Phe | Asp | Tyr | Asn | His | Ala |  |
|     | 530 |     |     |     |     | 535 |     |     |     |     | 540 |     |     |     |     |  |
| Phe | Ile | Lys | Ser | Ser | Phe | Phe | Asn | Ser | Ala | Ser | Asp | Val | Trp | Thr | Tyr |  |
| 545 |     |     |     |     | 550 |     |     |     |     | 555 |     |     |     |     | 560 |  |
| Gly | Phe | Gly | Ala | Asp | Ala | Leu | Tyr | Asn | Phe | Ile | Asn | Asp | Lys | Ala | Thr |  |
|     |     |     |     | 565 |     |     |     |     | 570 |     |     |     |     | 575 |     |  |
| Asn | Phe | Leu | Gly | Lys | Asn | Asn | Lys | Leu | Ser | Val | Gly | Leu | Phe | Gly | Gly |  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
|     |     |     | 580 |     |     |     |     | 585 |     |     |     |     | 590 |     |     |  |
| Ile | Ala | Leu | Ala | Gly | Thr | Ser | Trp | Leu | Asn | Ser | Glu | Tyr | Val | Asn | Leu |  |
|     |     | 595 |     |     |     |     | 600 |     |     |     |     | 605 |     |     |     |  |
| Ala | Thr | Val | Asn | Asn | Val | Tyr | Asn | Ala | Lys | Met | Asn | Val | Ala | Asn | Phe |  |
|     | 610 |     |     |     |     | 615 |     |     |     |     | 620 |     |     |     |     |  |
| Gln | Phe | Leu | Phe | Asn | Met | Gly | Val | Arg | Met | Asn | Leu | Ala | Arg | Pro | Lys |  |
| 625 |     |     |     | 630 |     |     |     |     |     | 635 |     |     |     |     | 640 |  |
| Lys | Lys | Asp | Ser | Asp | His | Ala | Ala | Gln | His | Gly | Ile | Glu | Leu | Gly | Leu |  |
|     |     |     | 645 |     |     |     |     | 650 |     |     |     |     |     | 655 |     |  |
| Lys | Ile | Pro | Thr | Ile | Asn | Thr | Asn | Tyr | Tyr | Ser | Phe | Met | Gly | Ala | Glu |  |
|     |     | 660 |     |     |     |     | 665 |     |     |     |     |     | 670 |     |     |  |
| Leu | Lys | Tyr | Arg | Arg | Leu | Tyr | Ser | Val | Tyr | Leu | Asn | Tyr | Val | Phe | Ala |  |
|     | 675 |     |     |     |     | 680 |     |     |     |     |     | 685 |     |     |     |  |
| Tyr |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |

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|-----|-----|-----|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
|     |     |     |       |     |     |     |     |     |     |     |     |     |     |     |     |  |
|     |     |     | <400> | 22  |     |     |     |     |     |     |     |     |     |     |     |  |
| Glu | Asp | Asp | Gly   | Phe | Tyr | Thr | Ser | Val | Gly | Tyr | Gln | Ile | Gly | Glu | Ala |  |
| 1   |     |     | 5     |     |     |     |     |     | 10  |     |     |     |     | 15  |     |  |
| Ala | Gln | Met | Val   | Thr | Asn | Thr | Lys | Gly | Ile | Gln | Glu | Leu | Ser | Asp | Asn |  |
|     |     | 20  |       |     |     |     | 25  |     |     |     |     | 30  |     |     |     |  |
| Tyr | Glu | Lys | Leu   | Asn | Asn | Leu | Leu | Asn | Ser | Tyr | Ser | Thr | Leu | Asn | Thr |  |
|     | 35  |     |       |     |     | 40  |     |     |     |     |     | 45  |     |     |     |  |
| Leu | Ile | Lys | Leu   | Ser | Ala | Asp | Pro | Ser | Ala | Val | Ser | Gly | Ala | Ile | Asn |  |
|     | 50  |     |       |     | 55  |     |     |     |     |     | 60  |     |     |     |     |  |
| Asn | Leu | Asn | Ala   | Gly | Ala | Thr | Gly | Leu | Leu | Lys | Glu | Lys | Thr | Asn | Ser |  |
| 65  |     |     |       | 70  |     |     |     |     |     | 75  |     |     |     |     | 80  |  |
| Pro | Ala | Tyr | Gln   | Ala | Val | Ser | Leu | Ala | Leu | Asn | Ala | Ala | Val | Gly | Leu |  |
|     |     |     | 85    |     |     |     |     | 90  |     |     |     |     |     | 95  |     |  |
| Trp | Asn | Thr | Ile   | Gly | Tyr | Ala | Val | Met | Cys | Gly | Asn | Gly | Asn | Gly | Thr |  |
|     |     | 100 |       |     |     |     |     | 105 |     |     |     |     | 110 |     |     |  |
| Ser | Asn | Gly | Pro   | Gly | Ser | Val | Ile | Phe | Asn | Gly | Glu | Pro | Gly | Gln | Gly |  |
|     | 115 |     |       |     |     | 120 |     |     |     |     |     | 125 |     |     |     |  |
| Ser | Thr | Gln | Ile   | Thr | Cys | Asn | Arg | Phe | Glu | Ser | Thr | Gly | Pro | Gly | His |  |
|     | 130 |     |       |     | 135 |     |     |     |     |     | 140 |     |     |     |     |  |
| Ser | Met | Ser | Ile   | Asp | Glu | Phe | Lys | Lys | Leu | Asn | Gln | Ala | Tyr | Gln | Ile |  |
| 145 |     |     |       | 150 |     |     |     |     |     | 155 |     |     |     |     | 160 |  |
| Ile | Gln | Gln | Ala   | Leu | Lys | Gly | Asn | Gly | Phe | Pro | Glu | Leu | Gly | Asn | Asn |  |
|     |     |     | 165   |     |     |     |     | 170 |     |     |     |     |     | 175 |     |  |
| Gly | Thr | Ser | Val   | Ser | Val | Glu | Tyr | Lys | Tyr | Glu | Cys | Lys | Gln | Asn | Asn |  |
|     |     | 180 |       |     |     |     | 185 |     |     |     |     |     | 190 |     |     |  |
| Asn | Asp | Ile | Asp   | Gly | Gly | Val | Arg | Gln | Phe | Cys | Gln | Ala | Lys | Asn | Gly |  |
|     | 195 |     |       |     |     | 200 |     |     |     |     |     | 205 |     |     |     |  |
| Ser | Ser | Ser | Gly   | Ser | Asn | Gly | Ser | Ser | Gly | Asn | Ser | Lys | Gln | Thr | Thr |  |
|     | 210 |     |       |     |     | 215 |     |     |     |     |     | 220 |     |     |     |  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Gln | Asp | Gly | Val | Thr | Ile | Thr | Thr | Thr | Asn | Ser | Ser | Asn | Glu | Ala |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |
| Thr | Val | Asn | Phe | Ser | Ile | Thr | Asn | Asn | Ala | Glu | Gln | Leu | Leu | Asn | Gln |
|     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |
| Ala | Ala | Asn | Ile | Met | Gln | Val | Leu | Asn | Thr | Gln | Cys | Pro | Leu | Val | Arg |
|     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |
| Ser | Thr | Asn | Asp | Glu | Asn | Ala | Pro | Gly | Gly | Gly | Gln | Pro | Trp | Gly | Leu |
|     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |
| Ser | Thr | Thr | Gly | Asn | Ala | Cys | Gln | Ile | Phe | Gln | Gln | Glu | Phe | Ser | Gln |
|     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |
| Val | Thr | Ser | Met | Ile | Lys | Asn | Ala | Gln | Glu | Ile | Val | Ala | Ser | Gln | Ile |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |
| Ala | Asn | Ala | Asn | Gln | Lys | Ala | Glu | Ile | Thr | Asn | Pro | Ser | Asn | Phe | Asn |
|     |     |     | 325 |     |     |     |     |     | 330 |     |     |     |     | 335 |     |
| Pro | Phe | Thr | Asp | Ala | Gly | Phe | Ala | Gln | Asn | Met | Leu | Lys | Asn | Ala | Arg |
|     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |
| Ala | Gln | Ala | Glu | Met | Phe | Asn | Leu | Ala | Glu | Gln | Val | Lys | Lys | Asn | Leu |
|     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |
| Glu | Val | Met | Gln | Asn | Asn | Asn | Asn | Val | Asn | Ala | Glu | Leu | Ser | Gly | Phe |
|     | 370 |     |     |     | 375 |     |     |     |     |     | 380 |     |     |     |     |
| Gly | Asp | Gly | Met | Thr | Asn | Phe | Val | Ser | Ala | Phe | Leu | Ala | Ser | Cys | Lys |
| 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     | 400 |     |
| Asn | Asp | Gly | Thr | Leu | Pro | Asn | Gln | Gly | Val | Thr | Ser | Asn | Thr | Trp | Gly |
|     |     |     | 405 |     |     |     |     |     | 410 |     |     |     |     | 415 |     |
| Ala | Gly | Cys | Ala | Tyr | Val | Gly | Glu | Thr | Ile | Thr | Ala | Leu | Asn | Asn | Ser |
|     |     |     | 420 |     |     |     |     | 425 |     |     |     |     | 430 |     |     |
| Ile | Ala | His | Phe | Gly | Thr | Gln | Ala | Glu | Gln | Ile | Glu | Gln | Ala | Glu | Asn |
|     |     | 435 |     |     |     |     | 440 |     |     |     |     | 445 |     |     |     |
| Ile | Ala | Asp | Thr | Leu | Val | His | Phe | Lys | Ser | Arg | Tyr | Asn | Glu | Leu | Gly |
|     | 450 |     |     |     |     | 455 |     |     |     |     | 460 |     |     |     |     |
| Asn | Thr | Tyr | Asn | Ser | Ile | Thr | Thr | Ala | Leu | Ser | Lys | Val | Pro | Asn | Ala |
| 465 |     |     |     | 470 |     |     |     |     |     | 475 |     |     |     |     | 480 |
| Gln | Ser | Leu | Asn | Val | Met | Gly | Lys | Lys | Asn | Asn | Pro | Tyr | Ser | Pro | Gly |
|     |     |     | 485 |     |     |     |     |     | 490 |     |     |     |     | 495 |     |
| Ile | Glu | Thr | Asn | Tyr | Tyr | Leu | Asn | Asn | Ser | Tyr | Asn | Gln | Ile | Gln | Thr |
|     |     |     | 500 |     |     |     |     | 505 |     |     |     |     | 510 |     |     |
| Ile | Asn | Gln | Glu | Leu | Gly | Arg | Asn | Pro | Phe | Arg | Lys | Val | Gly | Ile | Val |
|     |     | 515 |     |     |     |     | 520 |     |     |     |     | 525 |     |     |     |
| Ser | Ser | Gln | Thr | Asn | Asn | Gly | Ala | Met | Asn | Gly | Ile | Gly | Ile | Gln | Val |
|     | 530 |     |     |     | 535 |     |     |     |     |     | 540 |     |     |     |     |
| Gly | Tyr | Lys | Gln | Phe | Phe | Gly | Gln | Lys | Arg | Lys | Trp | Gly | Ala | Arg | Tyr |
| 545 |     |     |     |     | 550 |     |     |     |     | 555 |     |     |     |     | 560 |
| Tyr | Gly | Phe | Phe | Asp | Tyr | Asn | His | Ala | Phe | Ile | Lys | Ser | Ser | Phe | Phe |
|     |     |     |     | 565 |     |     |     |     | 570 |     |     |     |     | 575 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

Trp Leu Asn Ser Glu Tyr Val Asn Leu Ala Thr Met Asn Asn Val Tyr  
 625 630 635 640  
 Asn Ala Lys Met Asp Val Ala Asn Gln Phe Leu Phe Asn Met Gly Val  
 645 650 655  
 Arg Met Asn Leu Ala Arg Pro Lys Lys Lys Gly Ser Asp His Ala Ala  
 660 665 670  
 Gln His Gly Ile Glu Leu Gly Leu Lys Ile Pro Thr Ile Asn Thr Asn  
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<210> 36





